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INVESTIGATIONS ON BLACK KNOT OF PLUMS AND CHERRIES

IV. STUDIES IN PATHOGENICITY AND PATHOLOGICAL HISTOLOGY[1]

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In this article, concluding the present investigations on black knot, studies on pathogenicity and pathological histology of the host are the two major phases under consideration, while varietal susceptibility and certain experiments in control are dealt with in a minor way.

REVIEW OF LITERATURE

Farlow (*1*) in 1876 stated that "we have made direct experiments to show that the spores of the knot on the choke cherry will germinate and produce the knot in healthy plum trees." He did not cite the method by which these results were obtained. In 1913 Gilbert (*3*) reported some cross-inoculation experiments. He used the following methods: "(1) Spores sprayed from time to time on actively growing branches. (2) By means of finely-pointed glass tubing spores injected into stems at various depths from cambium region outward. (3) Incisions were made in the bark into which was placed a drop of culture medium containing germinating spores. (4) Small side branches were partly torn from the main stem and a spore suspension introduced into the wound. They were then covered with grafting wax or paraffin. Cross-inoculations were made between choke cherry, wild plum, cultivated plum and cherry using ascospores and conidia." He states that in no single instance was there the formation of any typical knot. As a check on these experiments inoculations were made upon the choke cherry using spores from other choke cherries, and normal knots were obtained. Gilbert did not cite which of the methods proved successful nor did he give his method of obtaining either pure cultures or suspensions of ascospores and conidia.

Humphrey (*5*) in 1893 reported numerous unsuccessful attempts to inoculate plums with pycnospores which he claimed to have obtained by culture from ascospores.

Stewart (*13*) in 1914 made a worthy contribution on the pathological anatomy of black knots. He sectioned knots in almost all stages of development and enumerated in careful detai the anatomical features of diseased and healthy tissues of *Prunus virginiana* L.

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PATHOGENICITY STUDIES

In order to ascertain the infection capabilities of ascospores and conidia of *D. morbosum*, and, if possible, some of the conditions governing infection, experiments involving more than 900 inoculations were conducted over a period of five years.

During the first year the majority of inoculations were made as follows. The surface of the branch was first sterilized with mercuric chloride (1 : 1000) and washed with sterile water after which the branch was injured to the cambium with a scalpel and the inoculum was inserted. The inoculated wound was then covered first with moist blotting paper and then with wax paper. All inoculations during this year (1930) were made on branches more than one year old and the inoculum used was in many cases a *Coniothyrium* sp., which was later proved not to be a pycnidial stage of *D. morbosum* (*8*).

In 1931 the method of inoculation was in some cases similar to that used during the previous year. In others the surface of uninjured current season twigs in protected positions on the trees were either atomized, or painted by means of a camel's hair brush, with aqueous suspensions of conidia from pure cultures of *D. morbosum*. In order to improve chances of infection when dry weather followed inoculation, twigs were in some cases atomized with spores a second and sometimes a third time.

In 1932 methods of inoculation were similar to those of the previous year with the exception that pure suspensions of ascospores of *D. morbosum* obtained by allowing mature perithecia of *D. morbosum* to eject their ascospores into water, were used as inoculum.

During the following two years two other methods were also employed. One of these was a modification of the method used by Keitt (*6*) in his peach scab investigations, by which wicks from Erlenmeyer flasks containing water were wound loosely around inoculated current season twigs. On other occasions "patch grafting"[3] was resorted to, by means of which small sections of unswollen host material taken from just beyond the borders of visible knot swellings where mycelium of *D. morbosum* was assumed to be present were grafted onto healthy branches. During May 1934 seventy-six grafts were made, some of which were from *P. domestica* onto the same host while others were made from *P. domestica* onto *P. cerasus* and sweet cherry. Branches of all ages were used in making these grafts.

The details of these experiments are summarized in Table 1.

An examination of Table 1 reveals the following facts. During 1930 only one knot developed as a result of inoculation by the first method. During 1931, when current season twigs were inoculated with conidia (1) during May, six typical knots were produced, and (2) during June, three knots developed. On all of these, conidia of *Hormodendrum* were produced and on eight of them perithecia of *D. morbosum* finally developed. All of the above-mentioned knots originated on current-season twigs and all were visible during the fall of the season of infection, conidia of *Hormodendrum* being produced on one during the fall and on the remaining eight the following spring. Mature perithecia of *D. morbosum* developed on one knot within a year after infection and within two years on seven others.

[3] R. V. Harris of East Malling Research Station kindly loaned his instruments (developed by Roach (*11*)) for these experiments.

TABLE 1.—SUMMARY OF BLACK KNOT INFECTION EXPERIMENTS IN WHICH POSITIVE RESULTS WERE OBTAINED

Inoculations			Inoculum used		Results	
Date	Material and method	Host	Source	Type		
13.6.30	Large branch injured, inoculum inserted; covered 14 days.	Reine Claude plum.	Fruiting surface of knot on Lombard plum.	Conidia of *Hormodendrum* sp. plus knot tissue.	20.9.30 31.8.31 1.8.32	Tissues swollen in vicinity of area of inoculation. Small area of swollen portion bearing conidia of *Hormodendrum sp.* (1/15)* Knot two inches in length bearing conidia.
9.4.31	Branches ½″–¾″ in diameter, injured, inoculum inserted; covered 14 days.	Lombard plum.	Monoascospore culture from knot, Lombard plum.	Conidia of *Hormodendrum* sp. plus knot tissue.	1.6.31 1.9.32	Three branches swollen with rough surface. Knot-like in appearance but no appearance of conidia.
22.5.31	Current season twigs, uncovered, inoculum painted on.	Lombard plum.	Monoascospore culture from knot, Lombard plum.	Conidia of *Hormodendrum* sp. plus knot tissue.	2.9.31 1.11.31 4.6.32 14.5.33	Small swelling on two twigs. Small swelling on three twigs (3/10) Knots producing conidia of *Hormodendrum* sp. on all three twigs. Mature perithecia of *D. morbosum* on two of the three knots.
1.5.31	Branches ½″–1″ in diameter injured, inoculum inserted, covered 12 days.	*P. cerasus.*	Monoconidial cultures from knot, Lombard plum.	Conidia *Hormodendrum* sp. plus *Coniothyrium* sp.	28.6.31 14.8.32	Swollen areas with rough surfaces on four inoculated branches. All branches swollen but no appearance of conidia.
21.5.31	Current season twigs, inoculum painted on.	Lombard plum.	Monoascospore cultures from knot, Lombard plum.	Conidia of *Hormodendrum* sp.	20.8.31 19.9.31 9.6.32 22.4.33	Small swelling on one twig. Small swelling on three twigs (3/10). Three knots producing conidia of *Hormodendrum* sp. Three knots producing perithecia of *D. morbosum.*

TABLE 1.—SUMMARY OF BLACK KNOT INFECTION EXPERIMENTS IN WHICH POSITIVE RESULTS WERE OBTAINED—*Concluded*

Inoculations			Inoculum used		Results	
Date	Material and method	Host	Source	Type		
12.6.31 14.6.31 18.6.31	Current season twigs, inoculum painted on.	Lombard plum.	Monoascospore cultures from knots Lombard plum.	Conidia.	1.9.31 19.4.32 15.6.32 1.5.33	Three knots developed on two twigs. Conidia of *Hormodendrum* sp. on one knot. (2/64).† Mature perithecia of *D. morbosum* on small fruiting area of one knot. Conidia on other two knots. Mature perithecia of *D. morbosum* on three knots.
7.5.32	Branches one year old bearing current season twigs; inoculum painted on.	Lombard plum.	Knot, Lombard plum.	Ascospores.	18.9.32 1.4.33	Knots appearing on two current-season twigs. Conidia of *Hormodendrum* sp. on both knots (2/10). Mature perithecia of *D. morbosum* on two knots.
2.5.32 10.5.32 15.5.32	Branches one year old bearing current season twigs; inoculum painted on.	Lombard plum.	Knot, Lombard plum.	Ascospores	1.9.32 14.6.33 15.5.34	One knot appearing on current-season twig (1/5). Conidia of *Hormodendrum* sp. on knot. No perithecia of *D. morbosum* developed on this knot.
7.5.32 10.5.32	Branches one year old bearing current season twigs; inoculum painted on.	Lombard plum.	Knot, Lombard plum.	Ascospores.	1.9.32 1.4.33	Two knots on current-season twigs, both producing conidia of *Hormodendrum* sp. (2/6). Perithecia of *D. morbosum* on one knot.
9.5.32	Branches one year old bearing current season twigs; inoculum painted on.	Reine Claude plum.	Monoascospore cultures from Lombard plum.	Conidia.	1.9.32 30.4.33	One knot on current season twig producing conidia (Plate I, figures 1–3). (1/5). Perithecia of *D. morbosum* on knot.

7.5.33	Current season twigs (Keitt's method, see text).	Reine Claude plum.	Monoascospore culture from Reine Claude plum.	Conidia.	25.9.33 Three knots on two twigs. All producing *Hormodendrum* conidia (2/6). 14.5.34 Perithecia of *D. morbosum* on two knots.
10.5.33	Current season twigs (Keitt's method, see text).	Lombard plum.	Monoascospore culture from Reine Claude plum.	Conidia of *Hormodendrum* sp.	25.8.33 One knot on twig (1/4). 4.9.33 Knot producing conidia of *Hormodendrum* sp. 14.5.34 No perithecia of *D. morbosum* on this knot.
10.5.33	Current season twigs (Keitt's method, see text).	Lombard plum.	Monoconidial culture from Lombard plum.	Conidia of *Hormodendrum* sp.	20.9.33 Five tiny knots on one twig (1/5). 1.9.34 No further development. (Small mechanical injury at base of twig.)
4.5.34	"Patch-grafting."	Lombard plum.	Unswollen bark from Reine Claude containing mycelium of *D. morbosum*.		15.8.34 Knots on three grafted branches, all of which produced conidia of *Hormodendrum* sp. (3/76).‡

*The numerator refers to the number of branches which developed typical black knot infection while the denominator refers to the number of branches or twigs inoculated in the series.

†Denominator refers to total number of inoculations made during June 1931, 1932 and 1933.

‡Denominator in this case refers to total number of patch-grafts made.

It will also be observed that when infection courts were provided on branches more than one year old on various hosts swellings developed in some cases though typical knots were never produced (Figure 1, A and B), (Plate I, 4). Some of these swellings had all the appearance of normal knots during their first year except that conidia were apparently not produced.

During 1932 when ascospores and conidia were used as inoculum on current season twigs as in the previous year six typical knots developed, of which five were produced as a result of inoculations with ascospores of *D. morbosum* and one as a result of inoculating with *Hormodendrum* conidia. Conidia of *Hormodendrum* sp. developed on all six knots and perithecia of *D. morbosum* developed on four. On five of these knots *Hormodendrum* conidia appeared during the fall of the year of infection and on four, perithecia of *D. morbosum* developed in less than one year after the inoculations were made.

During 1933 by the use of Keitt's method nine knots developed as a result of inoculating with *Hormodendrum* conidia. Five of these appeared on a single twig (Plate I, 5) during the fall of the year of infection but developed no further. The failure of these five knots to continue their development was directly ascribed to a mechanical injury which the base of the twig received during the following winter. The twig itself, though somewhat delayed in development the next spring, was not killed and the presence of the fungus in the swellings one year later was determined by sectioning. It has frequently been observed during the present investigations that a delicate balance exists between *D. morbosum* and the host, and that any serious disturbance of the growth of the host reflects unfavourably on the pathogen, frequently resulting in its death.

Perithecia of *D. morbosum* developed on 2 knots produced in 1933, within a year after inoculation.

In 1934 three knots developed on branches of Lombard plum as a result of "patch grafting". One of these is illustrated in Plate I, 6. Branches on which grafts were successful were two years old in two instances and considerably older in the other case.

It will be observed that perithecia of *D. morbosum* did not always develop on artificially produced knots which developed conidia. This was accounted for in most instances by parasitism of the stroma by *Cephalothecium roseum* though in a few cases it is possible that some other factor or factors may have prevented their formation.

From the numerical standpoint it will be observed that only a small percentage of the inoculated branches or twigs developed black knots. Out of a total of 622 branches or twigs inoculated with ascospores or conidia of *D. morbosum* 19 (3%) developed black knot infection. However, 16 (84%) of these twigs were inoculated during May and the remaining 3 were inoculated during early June. If percentage infection is calculated on the basis of number of twigs infected, 26% of the twigs inoculated during May developed one or more knots. On the other hand, if calculations are made on the basis of number of knots in proportion to number of inoculated twigs, 34% infection was obtained during May.

The outstanding features of the foregoing infection experiments may be summarized as follows: (1) Typical black knots were produced on

P. domestica inoculated with either ascospores of *D. morbosum*, conidia of the imperfect stage (*Hormodendrum* sp.) or host tissue containing mycelium of *D. morbosum;* (2) though branches of all ages were inoculated with spore suspensions of *D. morbosum* typical black knots developed only on current season twigs in all cases except one; and (3) though inoculations were carried out at all times of the year only those made during May and early June were successful.

It is realized that only partial success was obtained with any method of inoculation used in the foregoing infection experiments. The latter are open to the criticism that they were conducted outdoors where the inoculated twigs were unprotected from chance infection by the black knot pathogen from the unsprayed check trees. Since, however, all experiments, including controls were conducted on sprayed trees, in the majority of cases in a young orchard some distance from the unsprayed trees, and since in nearly all cases the only knots present on the trees were the knots which developed on inoculated twigs (several knots on a twig in some cases), it would seem highly improbable that any of them developed as the result of chance infection.

A Brief Consideration of Factors Involved in Black Knot Infection

A consideration of the factors governing infection of *Prunus* spp. by the black knot pathogen must include temperature, relative humidity, amount and type of inoculum, and stage of host development.

Temperature

The average temperatures for the first week subsequent to the inoculations summarized in Table 1 varied from 10.5° C. to 15° C. These temperatures are relatively low and while it has been shown that *D. morbosum* grows quite rapidly on culture media at temperatures even lower than those cited above, it was found that the organism also grew rapidly at much higher temperatures. Therefore, it cannot be expected that temperature is the factor limiting the period of infection to approximately one month of the year.

Relative Humidity

In those cases where infection occurred the relative humidity for the first week after inoculation was always high, the average varying from 76 to 90%. It can be assumed from this that relative humidity must be high for some days after inoculation, before infection is possible. But an examination of the meteorological records showed that at many other times of the year the relative humidity was high and the temperature apparently favourable for a considerable length of time after inoculations were made and yet no infection resulted. Consequently, relative humidity is unlikely to be the factor limiting black knot infections to approximately the month of May.

Amount and Type of Inoculum

So far as the infection experiments are concerned an abundance of fresh inoculum was always placed on the host tissues when the latter were inoculated. In nature it has been proved that at least from early spring until late fall an abundance of ascospores or conidia are available (7). Even during the winter chlamydospores are probably present in fair

abundance (*9*). Inoculum then cannot be the factor limiting infection to a short period of the year.

Condition of the Host.

In the Niagara peninsula the host is at the height of its activity during May and in some years also during the early part of June. During this time the tissues of current season twigs are succulent and immature. Under conditions prevailing in a plum orchard receiving the usual amount of cultivation, linear growth of current season twigs proceeds at a progressively slower rate from the latter part of June on. Beginning sometime in June, therefore, there is a gradually decreasing supply of meristematic host tissue available for infection, and since it has been shown both in article III of this series and in the infection studies of the present article that nearly all knots originate on rapidly-growing current season tissues, it would appear that the condition of the host more than any other single factor limits infection by *D. morbosum* to the spring of the year. More evidence to support this contention will be submitted in the studies on the pathological histology of the host.

Though temperature and relative humidity are probably not the factors limiting black knot infection on *P. domestica* to a relatively short period in the spring of the year, these factors are probably most important in a consideration of the epidemiology of black knot. During a certain early stage in the ontogeny of a plum twig it appears to pass through a period when it is susceptible to black knot infection. As the twig matures the tissues appear to become resistant. Temperature or relative humidity or a combination of both can undoubtedly prolong or shorten this period of susceptibility. For example, cool, damp weather during May delays growth and thus renders the host susceptible for a long period. In addition, these factors may limit the possibility of the germination of the spores, thus affecting the epidemiology of the disease, but they do not limit infection to a short period, because in the course of their ontogeny all twigs pass through a susceptible stage which in the Niagara peninsula occurs during May and early June. By way of example the almost complete lack of infection in 1934 can most likely be ascribed to the exceptionally dry May, when the relative humidity averaged only 58%.

VARIETAL SUSCEPTIBILITY

A wide variety of both wild and cultivated species of Prunus are susceptible to black knot. In Ontario the disease has been observed on the following wild hosts: *Prunus americana* Marsh, *P. virginiana* L. and *P. pennsylvanica* L. In the United States *P. chicasa* Michx., *P. Besseyi* Bailey, *P. maritima* Wang., *P. demissa* (Nutt.) Walp., *P. serotina* Ehrb., *P. emarginata* Walp., *P. melanocarpa* (Nels) Rydb., *P. nigra* Aib., *P. padus* L., *P. pumila* L., *P. triloba* L., *P. umbellata* Ell., and *P. subcordata* have also been reported (*12*) to be susceptible.

Of the cultivated varieties of plums all appear to be more or less suscept ble to black knot. In the Niagara peninsula Reine Claude and Lombard varieties are highly susceptible, while other varieties show varying degrees of resistance. General statements, however, pertaining to the resistance or susceptibility of Prunus varieties over wide areas cannot be made because a variety may be susceptible in one district and may appear

FIGURE 1—A. Swelling on large branch of *P. cerasus* 3 months after inoculation with conidia (*Hormodendrum* sp.) of *D. morbosum*. B. Swelling on trunk of *P. cerasus* produced as in A.

PLATE I.—1–3. Photographs of black knot produced by inoculating current season twig of *P. domestica* with conidia *(Hormodendrum)* of *D. morbosum*. 1. Knot $3\frac{1}{2}$ months after inoculation; photo taken 25.8.32. 2. Knot one year older; photo taken 20.8.33. 3. Knot 2 years and 3 months after inoculation; photo taken 24.8.34. 4. Swelling on large branch of sweet cherry produced as a result of inoculation with conidia of *Hormodendrum* sp. 5. Showing incipient stages of young knot on current season twig produced by inoculation with conidia using Keitt's method; photo taken approximately 3 months after inoculation (24.8.33). 6. Knot on *P. domestica* produced by "patch-grafting"; photo taken 4 months later (7.9.34).

PLATE II.—1–5. Cross sections of twigs of *Prunus domestica* infected with *D. morbosum.* 1. Showing increase in development of both xylem and tissues external to the cambium in infected portion of stem; arrows indicate aggregates of fungous hyphae; section cut 25.8.32, × 32. 2. As in 1; note large lacunae in various parts of infected xylem. Note infected medullary rays extending nearly to pith; × 32. 3 and 5. Showing mycelium of *D. morbosum* advancing in mature xylem during winter; note gum-filled trachea in 3 and intercellular nature of mycelium; sections cut 3.3.32, × 300. 4. Showing separation of xylem bundles as result of medullary ray infection; section cut 16.9.31; × 70.

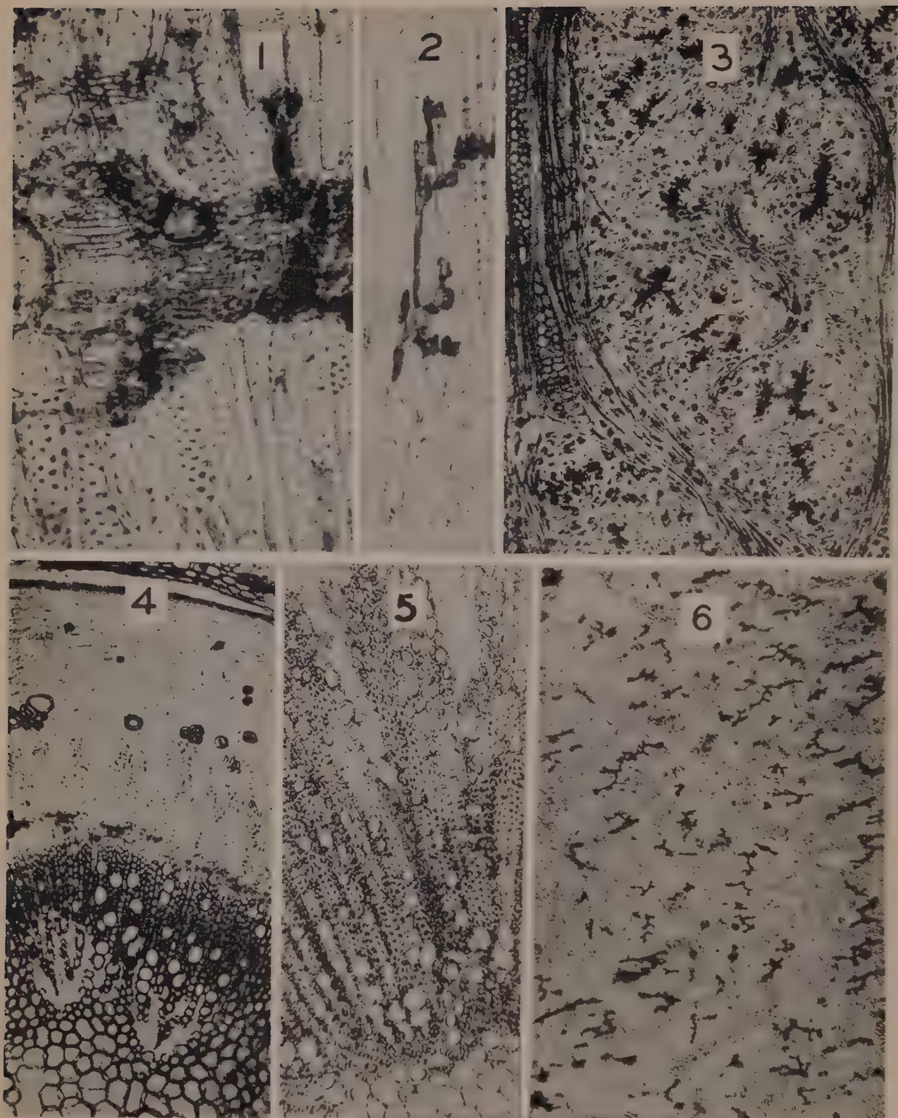

PLATE III.—1–6. Sections of normal and diseased tissues of *Prunus domestica*. 1. Longitudinal section at border of infected medullary ray; note bending of mature xylem elements and aggregation of hyphal strands; section cut 17.9.32, × 300. 2. Longitudinal section showing mycelium in mature xylem tissues in winter; section cut 5.3.32; × 300. 3. Tangential section showing normal multiseriate medullary rays at left and tremendous increase in size of infected ray; note black aggregates of hyphae interspersed in parenchyma; section cut 17.5.32; × 70. 4. Cross section of normal twig 2 mm. from visible knot; no mycelium present; section cut 7.6.32; × 70. 5. Cross section showing infection of medullary rays nearly to pith; section cut 25.8.32; × 70. 6. Section through infected cortex showing numerous aggregates of hyphae; section cut 20.4.32; × 30.

to be resistant in another district. For instance, the Montmorency variety of sour cherry has not been observed to be attacked by black knot in the Niagara peninsula, while on the other hand, this variety is attacked most severely in the Georgian Bay district. Likewise *P. serotina* Ehrb., a common variety in the Georgian Bay district, has never been observed to be attacked by black knot in that region, while Halsted (*4*) reports *P. serotina* to be highly susceptible in New Jersey. Examinations of sour cherries and plums growing adjacent to each other in the Niagara peninsula have frequently showed the plums to be affected with knot while the sour cherries were not observed to be attacked.

The observations recorded above would seem to indicate the existence of physiologic forms of *D. morbosum* not all of which are present in certain districts.

PATHOLOGICAL HISTOLOGY

Since, as stated above, Stewart (*13*) has already made a major contribution regarding the anatomy of black knots this phase will be dealt with in detail only in so far as necessary to consider certain phases and features of the pathological histology which Stewart did not investigate.

For this study fresh materials were utilized in all cases. Both healthy twigs and knots in all developmental stages ranging from freshly-inoculated twigs and scarcely-visible swellings to mature knots several years old were sectioned with a sliding microtome, stained in cotton blue and mounted in lacto-phenol. Sections were subjected at different times to preliminary microchemical tests the results of which appear incidentally in the following paragraphs.

The examination of twigs freshly-inoculated both in the laboratory and outdoors on *Prunus domestica* showed that while many of the spores germinated and penetrated the cuticle and sometimes several cell layers deeper, the majority appeared to develop no further. The germ tubes of many tapered sharply to a point and gradually disintegrated. Sections of branches one or more years old inoculated with conidia of *D. morbosum* showed in many cases that the conidia germinated and penetrated the periderm intercellularly to a maximum depth of approximately seven cell layers (Figure 2, C). It was concluded from these sections that the presence of numerous layers of periderm which cover branches one or more years old, with the exception (noted by Lodeman (*10*)) of small areas at the nodes tends to preclude infection by the black knot organism in such twigs.

Although the mycelium of the pathogen was always present in the cambial regions in small visible knots its passage through the intervening cortical tissues in the earlier stages of infection was never actually observed. This may be accounted for by the small proportion of successful infections resulting from penetration. Soon after the cambial region in current-season twigs is reached by the mycelium, hypertrophy and hyperplasia of the host tissues begin, and since the earliest visible swellings induced by infection have been observed on or close to August 15, probably between two and three months elapse until the organism induces anatomical changes in the cambial region sufficient to be visible externally. Sections of these incipient knots always revealed the presence of the mycelium of the fungus

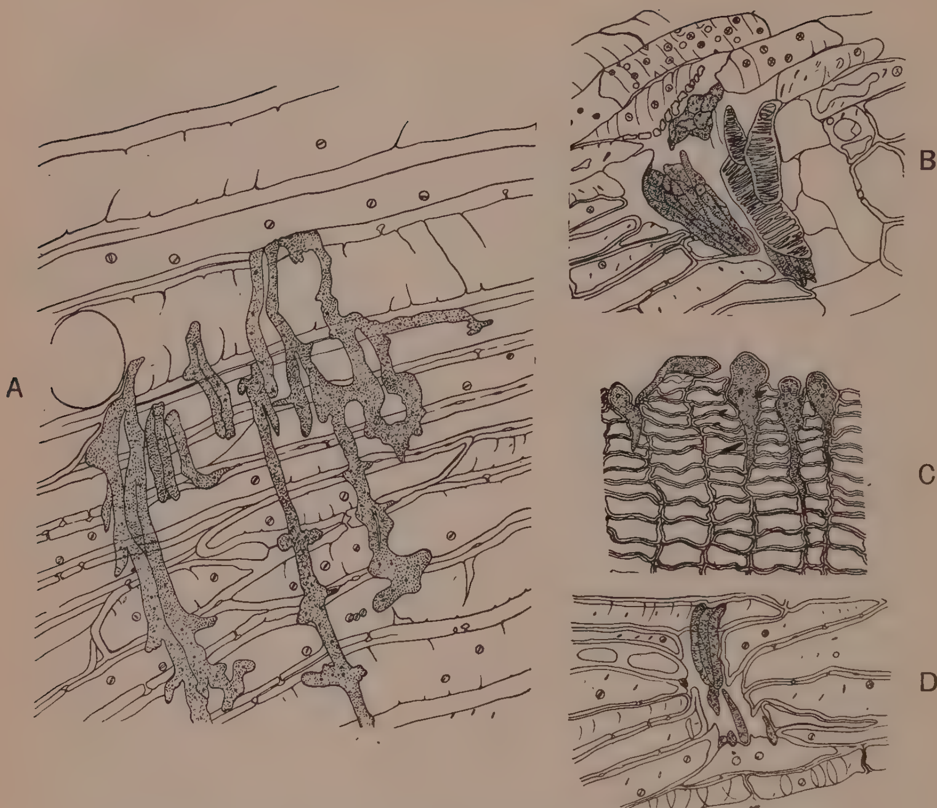

FIGURE 2—A. Showing mycelium of *D. morbosum* penetrating intercellularly, xylem tissues adjacent to infected medullary ray; note character of mycelium and method of progress; drawing from section cut 14.12.32; × 350. B. Showing mycelium of *D. morbosum* near end of infected medullary ray; note scalariform tracheids, the formation of which was induced by the pathogen; × 250. C. Long section of bark of 2-yr. old branch of *P. domestica* showing conidia which have germinated and penetrated the periderm intercellularly; traced from photo micrograph of section cut 15.6.31; × 500. D. As in B; × 250; note distortion of xylem elements.

in the medullary rays and frequently also in the cambial and cortical tissues. Hypertrophy and hyperplasia of host cells was most apparent in the vicinity of these medullary rays. The hyphal strands of the fungus in infections on current-season twigs have been frequently observed in the ray tissues nearly as deep as the pith (Plate II, 1 and 2) (Plate III, 5) but little advance in a radial direction was indicated after the ends of the rays opening into the cambium were invaded. Normal medullary rays in twigs of *P. domestica* are usually multiseriate and as soon as invasion of these rays occurs in the cambial region instead of forming the usual proportion of xylem elements and ray tracheids the cambium produces a relatively large number of parenchyma cells which are also many times their usual size (Plate III, 3). As time progresses these infected rays become so wide in the cambial region that finally the cambium separates and the component cells and groups of cells become displaced outwardly into the cortical tissues. In cross sections the mycelium of *D. morbosum* which

is always present in phalangial groups of hyphal strands (Plate II, 1, 2 and 4) (Plate III, 6) is observed to occupy a central position in infected rays (Plate II, 1 and 4). After the cambium is broken these strands in many cases extend unbroken from regions near the pith to the outer cortical tissues. Frequently only several rays on one side of the twig become infected. Since these widen out rapidly the twig soon becomes swollen and distorted on the infected side (Plate II, 1 and 2). Slow invasion of normal xylem and cortical tissues by the pathogen begins during the fall of the first season of infection. In these tissues as in all other host tissues the mycelium of the knot organism is intercellular and as it advances it pushes to either side the normal elements which are frequently much distorted as a result (Figure 2, A, B and D). The advancing tips of the mycelium in these cases are very characteristic in appearance, appearing almost plasmodial (Plate II, 3 and 5) (Plate III, 1 and 2).

In infected medullary rays the effect of the pathogen in stimulating cell development has frequently been observed at a distance of several cells from the mycelium but in no instance has dying of either the fungous mycelium or the adjacent host cells been observed during the season of infection. Microchemical tests have indicated the absence of starch from all infected medullary ray tissue at times when starch is present in corresponding normal rays. While normal rays possess a considerable number of cells with lignified walls corresponding tests of infected rays indicated a complete absence of lignin from all elements with the exception of the scalariform tracheids formed adjacent to the mycelium (Figure 2, B).

Numerous sections of knots of all ages have shown that not until after the hyphae of *D. morbosum* and the cells of its host have been in intimate contact for a period of approximately six or seven months does either host or parasite display any definite ill-effect as a result of the presence of the other. Sections, however; of knots on twigs less than one year old, made in January, February or later, indicate a gradual yellowing of the mycelium of the fungus accompanied by a browning of host cells in contact or nearby. Frequently also, nearby tracheae gradually became filled with gum (Plate II, 3). By April or May large gum pockets occasionally developed in the older infected host tissues (Plate II, 2) due to the dissolution of the parenchyma of the affected medullary rays. These were accompanied by a degeneration of the adjacent fungous mycelium. At this time in some instances mature perithecia were present on the exterior of such areas and in other cases an abundance of conidia were beginning to develop. Sections of secondary knots on branches more than one year old, where invasion had occurred as a result of the advance of the mycelium during the winter from the boundary of the old knot into normal tissues beyond, showed the same relation between host and parasite as mentioned above for primary knots on younger wood. In the meantime, subsequent to perithecial formation the tissues of the primary knot died.

Sections of both knots and adjacent normal twigs showed that during the summer months when the host is growing rapidly the mycelium of *D. morbosum* could not be found at a distance of even 2 mm. from the visible knot (Plate III, 4). Beginning with the advent of the dormant season, however, and continuing throughout the winter months the hyphae

of the fungus were found at increasing distances up to approximately three inches beyond the boundary of the visible knot. All areas of the stem invaded by the mycelium during the winter increased rapidly in size during May and began producing an abundance of conidia in June.

CONTROL EXPERIMENTS

The results of experiments in control up until 1932 have already been published (7). Since that time, however, spraying experiments have been continued with certain modifications of the original schedule. In blocks F, G and H (see (7) p. 584) consisting of 32 mature trees, Lombard variety, the most successful control experienced to date (average 96%, 2 years) was obtained by an application of lime sulphur 1 : 50 when the trees were in full bloom, in addition to the delayed dormant (1 : 8 lime sulphur) and the "shucks" spray (1 : 50 lime sulphur).

This improvement in control can be directly ascribed to the additional protection which the spray application at "full bloom" gave to the trees. It has already been determined that ascospores may be discharged under favourable temperature and moisture conditions at any period of the host development before buds burst until early June at which time all "shucks" have fallen. During the above-mentioned period growth is very rapid and newly-formed tissues being unprotected by the fungicide are exposed to infection. Therefore, if black knot is very severe in a plum orchard it would appear that a spray application when the trees are in full bloom in addition to the "delayed dormant" and "shuck" applications will give ample protection from the disease.

DISCUSSION

In this final article dealing with investigations on black knot certain additional information regarding the infection capabilities of *D. morbosum* and the changes induced in the host by this pathogen has been submitted. The progress of the disease has been carefully followed from the time when minute swellings representing incipient infections appeared three to four months after inoculation until the pathogen produced perithecia one or more years later. It was shown in article III of this series that more than 95% of black knots originated on current season wood. The results of infection experiments reported in the present paper confirm this finding. Although branches of *Prunus domestica* of all ages were inoculated with both ascospores and conidia of *D. morbosum* with a single exception typical knots developed on current season twigs only. Previous observations that nearly all natural infections can be distinguished in the fall of the year of infection have also been confirmed by the present studies. The time required by the pathogen to complete its life cycle varies. Knots which produced conidia during the fall of the year of infection in many cases produced perithecia the following winter, thus completing the life cycle of the pathogen within a year. On the other hand, knots which did not produce conidia until the following spring produced them in greater abundance than did the previous type of knot and perithecia did not appear until the second winter after infection.

Although infection experiments were conducted throughout the year only those made during May and early June gave positive results. While the period of susceptibility to infection in nature is possibly more extended

than this, nevertheless, all evidence indicates that the period during which infection can take place is very limited. The conclusion was reached that though temperature, relative humidity and amount and type of inoculum are important factors in the epidemiology of black knot, nevertheless, the condition of the host is the outstanding factor which limits infection to a relatively short period during the early part of the growing season. In the latter respect *D. morbosum* is analogous in its pathogenicity to *Taphrina deformans* (Fcl.) Tul., which as Fitzpatrick (*2*) has indicated is capable of infecting the immature leaf tissues of *Prunus persica* only during a very limited period. *D. morbosum*, on the other hand differs markedly in its pathogenicity from certain species of *Valsa* which have been found by Willison[4] of this laboratory to be capable of infecting *P. persica* only after the host is in a dormant condition.

A study of the pathological histology of the host revealed not only a strik ng hypertrophy and hyperplasia induced by the pathogen but also a specific and interesting relation between pathogen and host. *D. morbosum* apparently belongs to that group of pathogenic organisms which, displaying a high type of parasitism, live in intimate contact with their hosts for relatively long periods (usually six to eight months in the case of *D. morbosum*) with no apparent killing of either parasite or host tissue. In this respect *D. morbosum* would seem to resemble *T. deformans* and even the rust and smut fungi. The fact that *D. morbosum* is difficult to establish in culture when either ascospores or knot tissues in certain stages of development are used as sources of isolation also indicates a high type of parasitism.

In this the final paper of a series in connection with an extended study of black knot it would seem necessary to summarize the salient features in the life history of an organism which displays a more or less unique type of parasitism. Knots may be initiated in the spring or early summer by ascospores, conidia or possibly by chlamydospores. After three to four months these knots usually become visible as small swellings. Conidia may or may not be produced on the surface of the swollen host tissues in the fall. During the first season a *Coniothyrium* sp. invariably becomes associated with the pathogen. If conidia are produced by the pathogen during the fall, perithecia develop normally during the subsequent winter except in cases where the stroma has been parasitized by *Cephalothecium roseum* in which case the perithecial initials have been destroyed.

Secondary knots may arise as the result of mycelial invasion during the dormant season of healthy tissues beyond the borders of knots already formed. In the spring conidia are produced in great abundance on the surface of both primary and secondary knots which have not previously fruited. During midsummer the stroma of *D. morbosum* on almost all knots becomes parasitized by *C. roseum* and frequently also by numerous other fungi. During the winter perithecia of *D. morbosum* are produced on the majority of knots which have produced conidia during the summer. Knot tissues always die after having produced perithecia or after having been parasitized by other fungi. The above sequence of events continues until such time as the branch is finally girdled by the pathogen which also dies at that time.

[4] Willison, R. S. Unpublished data.

From the standpoint of control the investigations of the fundamental phases of the black knot problem have emphasized the fact that this disease is ocmparatively easy to control provided that the actively-growing tissues of the host are adequately protected with a fungicide during the relatively short critical period of infection. The formation of secondary knots can be prevented by the excision of primary ones during the growing season in which they are first observed.

In concluding it should be stated that there are many important facts regarding the black knot disease yet to be discovered. For example, a study of the infection capabilities of the pathogen on hosts more susceptible than *P. domestica* should yield especially interesting and valuable information regarding possible physiologic specialization.

SUMMARY

1. Various methods of inoculation of twigs and branches of plums and cherries with ascospores, conidia and mycelium of *Dibotryon morbosum* (Sch.) T. & S., are enumerated.

2. Only one knot resulted from the inoculation of branches more than one year old with diseased host tissue.

3. The majority of artifically-produced knots developed only on current season twigs inoculated with aqueous suspensions of either ascospores of *D. morbosum* or conidia of *Hormodendrum* sp., the imperfect stage.

4. In the aggregate only 3% of the inoculations using ascospores or conidia of *D. morbosum* gave positive results. Though inoculations were made throughout the year 84% of the knots produced resulted from inoculation during May. Twenty-six per cent infection was obtained in May.

5. Three knots were produced on old branches of *P. domestica* by "patch grafting" portions of unswollen host tissue taken from just beyond the border of knots onto healthy branches.

6. All artificially-produced knots were visible during the fall of the year of infection and many produced conidia of *Hormodendrum* sp. during the fall, and perithecia of *D. morbosum* during the following winter and spring, thus completing the life cycle of the pathogen within a year.

7. Though relative humidity and temperature are probably the most important factors governing the epidemiology of black knot, undoubtedly the condition of the host is responsible for the limitation of infection to a relatively short period in the early growing season.

8. The susceptibility of certain wild and cultivated species of Prunus to *D. morbosum* is discussed.

9. A study of the pathological histology of the host was made. Ascospores and conidia of *D. morbosum* germinate on the surface of healthy plum twigs and under favourable conditions mycelium reaches the cambium which is stimulated to produce abnormally large quantities of both xylem and phloem.

10. The pathogen is usually present in host tissues as compound phalangial strands of hyphae, chiefly radial and always intercellular. In the vicinity of invaded medullary rays the fungus induces an increase both in number and size of the parenchyma cells, and instead of the normal xylem elements scalariform tracheids are produced, frequently in contact with the mycelium.

11. After the growing season the fungus advances through normal xylem tissues and though it distorts the elements causes no visible hypertrophy during the winter.

12. *D. morbosum* exhibits a high type of parasitism, for host and parasite are capable of living in intimate contact with each other for approximately six or seven months before displaying any mutual ill effect.

13. Recent control experiments have indicated that excellent control of black knot can be obtained by an additional spray application of lime sulphur (1 : 50) during "full bloom" as well as the previously-recommended "delayed dormant" and "shuck" applications.

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Thanks are due Dr. G. H. Berkeley for many helpful suggestions offered at various times during the period of investigation.

REFERENCES

1. Farlow, W. G. Black knot. Bull. Bussey Inst. *24* : 440-453. 1876.
2. Fitzpatrick, R. E. The life history and parasitism of *Taphrina deformans*. Sci. Agr. *14* : 305-326. 1934.
3. Gilbert, E. M. Fungus-host relationship in black knot. Phytopathology *3* : 246-247. 1913.
4. Halsted, B. D. Destroy the black knot of plum and cherry. An appeal. N.J. Agr. Exp. Sta. Bull. *78* : 1-14. 1891.
5. Humphrey, J. E. The black knot of the plum. Mass. (Hatch) Agr. Exp. Sta. Rept. *10* : 235-238. 1893.
6. Keitt, G. W. Peach scab and its control. U.S.D.A. Bull. 395. 1917.
7. Koch, L. W. Investigations on the black knot of plums and cherries. I. Development and discharge of spores and experiments in control. Sci. Agr. *13* : 576-590. 1933.
8. ————. Investigations on the black knot of plums and cherries, II. The occurrence and significance of certain fungi found in association with *D. morbosum* (Sch.) T. & S. Sci. Agr. *15* : 80-95. 1934.
9. ————. Studies on the overwintering of certain fungi parasitic and saprophytic on fruit trees. Can. Jour. Research *11* : 190-206. 1934.
10. Lodeman, E. G. Black knot of plums and cherries, and methods of treatment. Cornell Univ. Agr. Exp. Sta. Bull. 81, 637-655. 1894.
11. Roach, W. A. Budding and grafting razors. Gardeners' Chronicle, March 24, 1934.
12. Seymour, A. B. Host index of the fungi of North America. Harvard University Press, p. 396. 1929.
13. Stewart, A., Some observations on the anatomy and other features of the black knot. Amer. Jour. Bot. *1* : 112-126. 1914.

Résumé

RECHERCHE SUR LE NŒUD NOIR (BLACK KNOT) DES PRUNIERS ET DES CERISIERS. L. W. Koch, Laboratoire fédéral de pathologie végétale, St. Catharines, Ont.

L'auteur énumère différents modes d'inoculation des rameaux et des branches de pruniers et de cerisiers avec des ascospores, des conidies et du mycélium de *Dibotryon morbosum* (Sch.) T. & S. L'inoculation, avec le tissus d'hôtes malades, de branches qui avaient plus d'un an, a provoqué l'apparition d'un seul nœud. La majorité des nœuds artificiellement produits ne se sont développés que sur des rameaux de la saison courante, inoculés de suspensions aqueuses d'ascospores de *D. morbosum* ou de conidies de l'espèce *Hormodendrum*, la phase imparfaite. Au total, seulement 3% des inoculations dans lesquelles on s'était servi d'ascospores ou de conidies de *D. morbosum* ont donné des résultats positifs. Quoique les inoculations aient été faites toute l'année, 84% des nœuds produits provenaient des inoculations faites en mai. Une infection de 26 pour cent a été obtenue en mai. Trois nœuds ont été produits sur de vieilles branches de *P. domestica* par le greffage en plaque, sur des branches saines, de parties non gonflées du tissu de l'hôte, prises juste au-delà de la bordure des nœuds, Tous les nœuds artificiellement produits étaient visibles pendant l'automne de l'année de l'infection et plusieurs ont produit des conidies de l'espèce *Hormodendrum* au cours de l'automne, et des périthécies de *D. morbosum* au cours de l'hiver et du printemps suivants, complétant ainsi le cycle évolutif du pathogène en une année. Quoique l'humidité relative et la température soient peut-être les facteurs les plus importants dans l'épidémiologie du nœud noir, il est évident que l'état de l'hôte est la cause de la limitation de l'infection à une période relativement courte, au commencement de la saison de végétation. La sensibilité de certaines espèces sauvages et cultivées de *Prunus* à *D. morbosum* est discutée. Une étude de l'histologie pathologique de l'hôte a été faite. Les ascospores et les conidies de *D. morbosum* germent à la surface de rameaux sains de pruniers; dans des conditions favorables, le mycélium atteint le cambium où il stimule la production de quantités anormales de xylème et de phloem. Dans les tissus de l'hôte, le pathogène est généralement présent sous forme d'hyphes phalangiennes, principalement radiales et toujours intercellulaires. Dans le voisinage des rayons médullaires envahis, le champignon provoque une augmentation du nombre et de la grosseur des cellules de parenchyme, et au lieu des éléments normaux de xyulème il se forme des trachéides scalariformes, fréquemment en contact avec le mycélium. Après la saison de végétation, le champignon pénètre à travers les tissus normaux de xylème et en dérange les éléments sans causer d'hypertrophie visible pendant l'hiver. Le *D. morbosum* exhibe un haut degré de parasitisme, car l'hôte et le parasite peuvent vivre en contact intime l'un avec l'autre pendant environ six ou sept mois sans manifester de mauvais effets mutuels. Il a été démontré par des expériences récentes que l'on peut fort bien prévenir le nœud noir par une pulvérisation supplémentaire de chaux soufrée (1:50) pendant la période de pleine floraison, ainsi que les applications déjà recommandées "dormantes retardée" et des "pétales".

EXPERIMENTS ON THE CONTROL OF CEREAL SMUTS BY SEED TREATMENT[1]

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Many of the varieties of wheat, oats, and barley grown in Canada are susceptible to smut. Accurate estimates of losses from smut diseases are difficult to obtain, but for the period 1920–1923 the average annual loss in this country was placed at $12,831,000. It is probable that much of the seed grain used during this period was treated before being sown. Had seed treatment of some kind not been practised the losses occasioned by the smut fungi would have been even more severe. It is equally evident that great possibilities still exist of reducing smut losses through improvements in seed treatments, and by the introduction of suitable smut-resistant varieties.

The ideal seed disinfectant should be effective, relatively cheap, easily applied, and yet not cause seed injury. It would be possible to draw up a long list of substances which are extremely toxic to smut spores, but for certain reasons most of them would be of little use for treating seed grain.

Until about 1896, copper sulphate, commonly known as bluestone, was probably the most widely used of all seed disinfectants. Formalin was then introduced, and it soon became the standard treatment for bunt of wheat, covered smut of barley, and the smuts of oats. In recent years copper and mercury dusts have come into general use for seed-treating purposes. Such substances are effective fungicides, are easily applied to the seed, and usually have a favourable effect on its germination.

Before any substance can be recommended to farmers for smut control it must be subjected to field tests under conditions suitable for natural smut infection. During the past five years tests of this kind have been made at the Dominion Rust Research Laboratory with no less than 37 substances. The results of some of these tests are not of sufficient importance to justify their publication. Others, obtained with some of the newer preparations now on sale in this or in other countries, may be of interest to agriculturists for purposes of reference. With this object in view these results have been summarized in the following tables, together with those from similar tests made with well-known seed disinfectants such as formalin and copper carbonate.

METHODS

The effectiveness of any smut treatment can be judged by comparing the percentage infection in treated and untreated rows of grain grown in the field. If infection in the untreated checks is relatively low it may not be possible to draw any conclusions from the results of the treatments. If, however, high infection has been secured in the untreated checks the results of the treatments tend to become significant.

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The conditions governing smut-infection have been studied by a number of workers, and those factors of greatest importance are now fairly well understood. As a result of this work it may be stated that, under field conditions, soil temperature, spore load, and the susceptibility of the host plant will to a great extent determine the degree of smut infection.

Temperature

Munerati (*5*, *6*) Faris (*2*), and others have shown that relatively low temperatures (10°–15° C.) favour bunt infection. For covered smut of oats the optimum temperature for infection was found by Reed and Faris (*7*) to be between 20° and 25° C., the exact point depending upon the moisture content of the soil and certain other factors. Faris (*1*) secured high infection with covered smut of barley at 10°, 15° and 20° C. when the soil moisture was kept at 40% or 50%. These results indicate that, under field conditions, early seeding of spring wheat, while the soil temperature is still low, should give high bunt infection, whereas a somewhat later seeding might be expected to give the best infection with the smuts of oats and covered smut of barley.

In practice it has been found that the date of the first seeding of spring wheat at Winnipeg is usually determined by the physical condition of the soil rather than by the soil temperature. In the period 1930–1934, during which the experiments referred to in this paper were conducted, the first date of seeding has fluctuated between April 21 in 1931, and April 29 in 1932. Perhaps the effect of date of seeding on smut infection may best be illustrated by some results obtained at Winnipeg in 1934 when smutted seed of wheat, oats, and barley was sown on five different dates, commencing April 28. On each of these dates four rod-rows were sown with each lot of smutted seed. The percentages of smut, recorded in Table 1, were arrived at by examining 200 heads in each of the four rows. It is evident from these results that spring wheat must be sown early if high bunt infection is to be obtained, whereas relatively late seeding may be expected to give good infection with the smuts of oats and covered smut of barley.

TABLE 1.—INFLUENCE OF THE DATE OF SEEDING ON SMUT INFECTION AT WINNIPEG IN 1934

Host plant	Smut	Per cent smut				
		Sown Apr. 28	Sown May 5	Sown May 14	Sown May 21	Sown June 2
Marquis wheat	*Tilletia laevis*	25.9	10.4	0.4	0.1	0
Kota wheat	*Tilletia tritici*	37.4	10.3	2.3	0.1	0
Trebi barley	*Ustilago hordei*	3.1	1.4	0.8	1.1	7.0
Abundance oats	*Ustilago avenae*	15.9	12.9	3.5	5.1	28.9
bundance oats	*Ustilago levis*	17.8	7.3	2.5	6.6	12.3

It should be pointed out that very little rain fell between May 5 and June 2 and, as a consequence, the soil became extremely dry. During this period, low soil moisture rather than unfavourable temperature was responsible for the poor infection on oats and barley. On June 5, 1.93 inches of rain fell, and good infection was secured in the rows of oats and barley sown on June 2, although wheat sown on the same date failed to become infected.

The soil temperature at this time must have been still favourable for infection by the smuts of oats and barley, although too high to permit infection by bunt of wheat.

Spore Load

The experiments of Heald and others (*3*, *4*) have shown clearly that if other conditions are kept constant smut infection varies with the number of spores adhering to the seed, or as it is usually termed, the spore load. When a susceptible variety of wheat is grown under conditions suitable for infection, a spore load of 1–200 (1 gram of spores to 200 grams of seed) will give very nearly maximum infection. With this rate of smutting approximately 36,000 spores adhere to each grain of wheat.

In the experiments, the results of which are summarized in this paper, two spore loads were used for wheat, namely, 1–200, and 1–1000. The species of bunt used was *Tilletia tritici*. The spore load for barley was 1–200. Oats were smutted at the rate of 1–500, except in the years 1930 and 1931 when a spore load of 1–1000 was used. The inoculum used in all of the experiments was prepared by crushing smutted heads in a mortar and sifting the resulting material through a 200-mesh sieve. In this way smut spores free from all but a trace of debris were obtained. All of these spore loads provided heavier contaminations than are usually found on seed grain. Nevertheless, the infection secured in the untreated rows of barley was less than that frequently observed in farmers' fields.

Varieties

The varieties selected for the experiments were all known to be susceptible to the particular physiologic forms of smut used as inoculum. They were as follows: Kota and Mindum wheat, Trebi barley, Longfellow oats in 1930, and Abundance oats in the years 1931-34.

Treatments

The dust treatments were applied to the inoculated seed usually the day before seeding. The rates of application are given in the following tables in ounces per bushel.

The only wet treatments employed were those with formalin and Clarke's Wheat Protector. These treatments were made on the day of seeding. The formalin solution was made up to the usual concentration so as to contain one part by volume of commercial formalin in 320 parts of solution. This is equivalent to one pound of formalin in about 30 gallons of water. The "sprinkle" treatments were carried out by adding this solution to the seed at the rate of about three-quarters of a gallon per bushel, after which the seed was covered for 4 hours. In treating barley by the "immersion" method the seed was poured into the solution. This caused many of the smut spores to float to the surface. The liquid was then drained off, and the seed was removed from the vessel and covered for 4 hours. The solution containing Clarke's Wheat Protector was applied to the seed at the rate of three-quarters of a quart per bushel.

The results obtained with the following seed disinfectants are summarized in this paper:—

1. Formalin (Standard Chemical Co., Montreal, P.Q.).

2. Deloro copper carbonate, containing 20% copper (Deloro Chemical Co., Deloro, Ont.).

3. Mococo copper carbonate, containing 54% copper (Mountain Copper Co., San Francisco, Cal.).

4. Copper sulphate dust.

5. Monohydrated copper sulphate (Nichols Copper Co., New York).

6. Basic copper chloride, containing 20% copper (Canadian Industries Ltd., Montreal, P.Q.).

7. Basic copper chloride, containing 48% copper (Canadian Industries Ltd., Montreal, P.Q.).

8. Copper sulphocyanate, containing 48% copper (Canadian Industries Ltd., Montreal, P.Q.).

9. Wackerite Dry Dusting Powder, containing 25% copper (Gesellschaft für elektrochemische Industrie, München, Germany).

10. Vitrioline, containing 17% copper (Usines Schloesing Frères and Cie., Marseilles, France).

11. Preparations manufactured by the Ansbacher Siegel Corp., New York: (1) Bordeaux dust, containing 12.5% copper. (2) Oxo Bordeaux dust containing 12.5% copper. (3) Colloidal Calomel. (4) Colloidal Yellow Oxide of Mercury.

12. Ceresan, containing 2% ethyl mercury chloride, (Bayer-Semesan Co., Wilmington, Del.).

13. New Improved Ceresan, containing 5% ethyl mercury phosphate (Bayer-Semesan Co., Wilmington, Del.).

14. Lunasan (Lunevale Products Ltd., Lancaster, Eng.).

15. Clarke's Wheat Protector (G. B. Clarke, Woburn Sands, Eng.).

16. Smuttox (Grasselli Chemical Co., Cleveland, Ohio).

Seeding and Smut Counts

The lots of treated and untreated seed were sown in the field in rod-rows replicated four times.

Smut infection was estimated about the time the grain began to ripen by examining 100 heads at two places in each row. Since four rows were sown with each lot of seed, the mean smut percentages recorded in the following tables are each based upon an examination of 800 heads.

RESULTS

Wheat

The results of the experiments on the control of bunt in artificially-smutted seed are summarized in Table 2.

The extent to which growing conditions may influence bunt infection is brought out by a comparison of the percentages of bunt occurring from year to year in plots of the same variety of wheat grown from untreated seed smutted with a uniform spore load. Soil temperature at the time of seed germination is probably the factor mainly responsible for these yearly fluctuations in bunt infection.

The infections obtained in the untreated rows provide also a good illustration of the effect of spore load on bunt infection. For the five-year period under consideration, a spore load of 1–1000 gave 20% infection on Kota and 38% on Mindum, whereas a 1–200 spore-load gave infections on these varieties of 58% and 64% respectively.

TABLE 2.—CONTROL OF BUNT (*Tilletia tritici*) IN KOTA AND MINDUM WHEAT BY SEED TREATMENT (ARTIFICIALLY-SMUTTED SEED; WINNIPEG, 1930–1934)

Treatment		Per cent bunt																			
		Kota										Mindum									
		Spore load 1–1000					Spore load 1–200					Spore load 1–1000					Spore load 1–200				
		1930	1931	1932	1933	1934	1930	1931	1932	1933	1934	1930	1931	1932	1933	1934	1930	1931	1932	1933	1934
Untreated		15.6	28.1	26.4	9.6	17.6	58.5	87.3	45.8	35.3	63.6	35.1	80.0	38.5	12.0	24.3	64.8	92.6	68.9	31.8	60.5
Formalin (1–320 sprinkle)		3.1	0	0.1	0	0	3.7	0.1	0.3	0	0	6.4	0.4	0.1	0.4	0	4.3	0.4	0.4	0	0.1
Copper carbonate (20% Cu.)	2 oz.	0.3	2.1		0.1	1.4	10.0	12.0		0.9	4.9	0.1	4.6		0.5	0.8	7.8	24.4		2.1	20.6
Copper carbonate (20% Cu.)	3 oz.	0.4	2.6				10.9	11.8				0.1	2.8				6.1	20.6			
Copper carbonate (54% Cu.)	2 oz.	1.0	0.9		0.1		9.9	6.1		0.5		0.9	0.3		0.1		13.5	23.4		0.4	
Copper carbonate (54% Cu.)	3 oz.	0.4	0.5	0.4			3.8	3.8	1.4			0.5	0.6	0.6			5.6	13.6	0.4		
Copper sulphate dust	2 oz.		2.6					5.3					4.5					17.8			
Copper sulphate dust (monohydrated)	2 oz.	1.8					8.4					0.3					2.6				
Basic copper chloride (20% Cu.)	2 oz.				0.8					3.3					0.1					4.4	
Basic copper chloride (20% Cu.)	3 oz.			0.3					7.8					3.0					8.9		
Basic copper chloride (48% Cu.)	2 oz.				0					2.4					0.1					2.8	
Basic copper chloride (48% Cu.)	3 oz.			0.5					2.4					0.5					2.1		
Copper sulphocyanate (48% Cu.)	2 oz.				1.6					12.5					2.5					15.8	
Wackerite Dry Dusting Powder	2 oz.					0.9					7.1					1.5					10.4
Vitrioline	2 oz.	1.4					16.8					0.3					18.9				
Vitrioline	3 oz.	0.6					12.6					0.6					17.4				
Bordeaux dust (12.5% Cu.)	2 oz.																11.0				
Oxo Bordeaux dust (12.5% Cu.)	2 oz.																14.9				
Colloidal calomel	2 oz.																58.9				
Colloidal yellow oxide of mercury	2 oz.																54.8				
Ceresan	2 oz.		0.9		0			7.4		0.1			4.1		0.1			15.3		0.1	
Ceresan	3 oz.		0.9	0.8				12.8	3.9				3.0	0.3				9.1	2.1		
New Improved Ceresan	½ oz.				0.1	0				0.4	1.6				0	0.1				0	3.9
Lunasan	2 oz.					0.6					5.8					1.0					14.0
Clarke's Wheat Protector					0					0.1					0					0.3	

By referring to the results in Table 2, it will be noticed that when bunt infection in the untreated plots was light, most of the treatments gave good control. With heavy infection in the untreated plots, however, control became more difficult, and differences in the effectiveness of treatments became apparent. In farm practice, wheat carrying a spore load of 1–200 would probably never be sown, but in experimental work heavy spore loads provide a useful method of evaluating the relative effectiveness of different seed-treating substances.

With few exceptions, the formalin treatment gave satisfactory results. But it must be borne in mind that the effectiveness of a liquid seed disinfectant such as formalin is determined not only by its fungicidal properties, but also by the manner in which it is applied. In the laboratory, where only a small quantity of grain has to be treated, it is a comparatively easy matter to standardize the treatment. On the farm, however, large quantities of grain must be treated at a busy time of the year, and the difficulties in the way of proper treatment are very great. For this reason, the results obtained with formalin in actual farm practice may fall far short of those secured in field-plot experiments.

Clarke's Wheat Protector, a liquid treatment, gave satisfactory results in the one year in which it was tested. However, it does not appear to have any particular advantage over the ordinary formalin treatment.

Many of the dusts when used with lightly-smutted seed reduced the bunt to one per cent or less, but they were not so effective when used with heavily-smutted seed.

In the tests conducted in 1933 and 1934 with New Improved Ceresan, this dust, applied at the rate of only ½ oz. per bushel, gave uniformly good bunt control, even when the seed was heavily smutted. It is a matter of considerable interest that such a light application of dust should protect the seed against bunt infection. Seed grain to which dust has been applied

Table 3.—Control of covered smut of Trebi barley by seed treatment (artificially-smutted seed; Winnipeg, 1931-1934)

Treatment		Per cent smut			
		1931	1932	1933	1934
Untreated		6.1	17.8	7.5	6.5
Formalin (1–320 sprinkle)		0	2.3	0.6	0
Formalin (1–320 immersion)		0	0	0.1	
Smuttox	3 oz.	4.1			
Copper carbonate (20% Cu.)	2 oz.	2.6			
Copper carbonate (20% Cu.)	3 oz.	1.4		8.4	
Copper carbonate (54% Cu.)	2 oz.	1.9			
Copper carbonate (54% Cu.)	3 oz.	1.9	9.3		
Basic copper chloride (20% Cu.)	2 oz.		16.3		
Basic copper chloride (20% Cu.)	3 oz.		13.1	8.1	
Basic copper chloride (48% Cu.)	2 oz.		4.6		
Basic copper chloride (48% Cu.)	3 oz.		5.6	1.3	
Copper sulphocyanate (48% Cu.)	3 oz.			9.6	
Wackerite Dry Dusting Powder	2 oz.				1.3
Ceresan	3 oz.	0.1	0.1	0.1	
New Improved Ceresan	½ oz.			0	0
Lunasan	2 oz.				0

at the rate of from 2 to 3 ozs. per bushel is unpleasant to handle, and under certain conditions may cause clogging of the drill. These objections are not met with in dusts which are effective when applied in relatively small amounts.

Barley

The results of experiments with covered smut of barley are summarized in Table 3. This smut was almost eliminated by immersing the seed in a solution of formalin. In 1931 naturally-smutted seed of barley containing many smut balls was treated in the same way. The plot grown from the treated seed was free from smut, whereas 9.1% smut was present in the plot grown from the untreated seed. The dust treatments with Ceresan, New Improved Ceresan, and Lunasan, reported in Table 3, gave equally good results, but the copper dusts proved to be unsatisfactory.

Oats

With the exception of Smuttox, a formalin-containing dust, all of the substances tested for the control of the oat smuts gave satisfactory results. By referring to the data given in Table 4 it will be evident that seed treatment controls loose smut as well as covered smut.

TABLE 4—CONTROL OF THE SMUTS OF OATS BY SEED TREATMENT (ARTIFICIALLY-SMUTTED SEED; WINNIPEG, 1930–1934)

Treatment		Per cent smut							
		Loose smut			Covered smut				
		1930	1931	1932	1930	1931	1932	1933	1934
Untreated		15.2	17.0	29.8	26.2	38.4	29.3	4.5	2.1
Formalin (1–320 sprinkle)		2.4	0	0	2.9	0	0	0	0
Smuttox	3 oz.	1.8	8.5		3.3	27.9			
Ceresan	3 oz.	0.1	1.3	0	1.0	1.6	0	0	
New Improved Ceresan	½ oz.							0	0.3
Lunasan	2 oz.								0

NOTE.—In 1930 the tests were made with Longfellow oats, and in the remaining years with Abundance.

In other trials, not reported in Table 4, copper dusts, such as copper carbonate and copper chloride, were found to be of little value for controlling the oat smuts. This finding corroborates the experience of other workers, that copper dusts do not effectively control the smuts of coarse grains.

In the discussion of bunt of wheat, it was pointed out that under farm conditions the difficulties of properly applying the formalin treatment tended to impair its effectiveness. The same objection applies to the treatment of oats and barley with formalin. This view is supported by reports from farmers of heavy smut infection in fields of oats and barley grown from formalin-treated seed. As compared with liquid treatment, the dusting of grain is an extremely simple process. For this reason the general use of good dust disinfectants may be expected to reduce greatly losses from the smuts of oats and barley, as well as those from bunt of wheat.

Because of the light infections in the untreated plots in 1933 and 1934, the results of the tests with New Improved Ceresan, as given in

TABLE 5.—CONTROL OF SMUT OF OATS BY SEED TREATMENT (NATURALLY-SMUTTED SEED; WINNIPEG, 1933).

Treatment		Per cent smut	
		Lot 1	Lot 2
Untreated		23.5	30.8
Formalin (1–320 sprinkle)		0.1	0.6
Ceresan	3 oz.	0	0.3
New Improved Ceresan	½ oz.	1.6	0.1

Table 4, are not of great value. This dust was used in some other experiments made in 1933 with two lots of naturally-smutted oats sent to the laboratory by farmers. In these tests, the results of which are shown in Table 5, New Improved Ceresan, when applied at the rate of ½ oz. per bushel, gave satisfactory smut control.

SUMMARY

A brief reference is made to improvements which have been effected in seed disinfectants used for the control of cereal smuts. Organic mercury dusts, because of their effectiveness and the ease with which they can be applied to seed, appear to be replacing liquid treatments and the copper dusts.

During the period 1930–1934, 37 preparations were tested in field experiments for the control of smut. The results obtained with 16 of these preparations are summarized in tables.

Formalin gave good control of bunt of wheat, covered smut of barley, and the smuts of oats. This treatment is relatively cheap, but it is more difficult to apply than the dust treatments, and under certain conditions may cause seed injury. The copper dusts are to be recommended only for treatment of wheat and hulless oats. In general, they gave good control of bunt when the seed was not too heavily contaminated with spores. New Improved Ceresan, an organic mercury dust, was effective in controlling bunt of wheat, covered smut of barley, and the smuts of oats. This dust, because of the light rate at which it is applied, should not cause clogging of drills. In certain experiments, the results of which are not recorded in this paper, seed treated with copper and mercury dusts gave a higher percentage of seedling emergence than untreated seed.

LITERATURE CITED

1. FARIS, J. A. Factors influencing the infection of *Hordeum sativum* by *Ustilago Hordei*. Amer. Journ. Bot. *11* : 189-214. 1924.
2. ————. Factors influencing the infection of wheat by *Tilletia tritici* and *Tilletia laevis*. Mycologia *16* : 259-282. 1924.
3. HEALD, F. D. The relation of spore load to the per cent of stinking smut appearing in the crop. Phytopath. *11* : 269-278. 1921.
4. ————, and L. W. BOYLE. Further notes on the relation of the spore load to the per cent of stinking smut appearing in the crop. Phytopath. *13* : 334-337. 1923.
5. MUNERATI, O. Osservazioni sulla recettivita del frumento per la carie. Atti. Accad. Lincei Cl. Sci. Fis. *V. 31* : 125-129. 1922.
6. ————. La basse temperature al momento della germinazione fanno sfuggire il grano all'attacco della carie? Atti. Accad. Lincei Cl. Sci. Fis. *V. 32* : 285-289, 1923.
7. REED, G. M. and J. A. FARIS. Influence of environmental factors on the infection of sorghum and oats by smuts. II. Experiments with the covered smut of oats and general considerations. Amer. Journ. Bot. *11* : 579-599. 1924.

Résumé

EXPÉRIENCES SUR LA POSSIBILITÉ DE PRÉVENIR LES CHARBONS DES CÉRÉALES PAR LE TRAITEMENT DE LA SEMENCE. W. F. Hanna et W. Popp, Laboratoire fédéral de recherches sur la rouille, Winnipeg, Man.

Les auteurs relatent sommairement les améliorations qui ont été apportées aux désinfectants employés sur la semence pour prévenir le charbon des céréales. En raison de leur utilité et de la facilité avec laquelle elles peuvent être appliquées, les poussières organiques de mercure paraissent devoir supplanter les traitements liquides et les poussières de cuivre. Pendant la période 1930-1934, il a été essayé 37 préparations dans des essais pratiques pour maîtriser le charbon. Les résultats donnés par 16 de ces préparations sont résumés en tableaux. La formaline s'est montrée efficace contre la carie du blé, le charbon vêtu de l'orge et le charbon de l'avoine. Ce traitement est relativement bon marché, mais il s'applique plus difficilement que les poussières et il peut même abîmer la semence dans certaines conditions. Les poussières de cuivre ne sont recommandées que pour le traitement du blé et de l'avoine nue. En général, elles ont bien maîtrisée la carie lorsque la semence n'était pas très contaminée de spores. Le nouveau Ceresan Amélioré, une poussière organique de mercure, a réussi à prévenir la carie du blé, le charbon vêtu de l'orge et le charbon de l'avoine. Cette poussière ne devrait pas boucher les tuyaux du semoir, car elle s'applique en faible quantité. Dans certaines expériences, dont les résultats ne sont pas enregistrés dans cet article, la semence traitée avec des poussières de cuivre et de mercure a donné un pourcentage plus considérable de plantules que la semence non traitée.

A STUDY OF THE PROTEIN REQUIREMENTS OF GROWING CHICKS[1]

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To grow chicks economically, it is essential that protein, a vital and expensive nutrient, be present in optimal amount. Since a study of the protein requirements involves certain aspects of avian metabolism as yet inadequately understood, the work reported here may be regarded in the light of a preliminary study only.

Nutritional workers have known for some years that equivalent amounts of protein from different sources do not have the same dietary value. Certain native proteins are known to be deficient in some essential amino acids and various tables of the biological value of proteins, based on feeding trials with mammals, have been elaborated. Many investigators in the field of avian protein requirements have almost wholly disregarded this aspect of the problem and have advocated protein levels for chick rations based on experiments in which a single type of protein was fed. Until such time as a purified diet, capable of satisfying the nutritive requirements of chicks in all factors, other than protein, is found, it is impossible to formulate absolute feeding values of proteins in avian nutrition. Experiments conducted in this laboratory have shown that a combination of proteins gave better growth and development than any single protein, and moreover that equivalent amounts of protein from either buttermilk powder, fish meal or meat meal did not give the same growth response. In view of these facts it seemed feasible to use a combination of proteins in this experiment rather than have results mitigated by some possible amino acid deficiency, or other, as yet unknown, factors.

Mussehl and Gish (*7*) reported that growth was progressively increased as the amount of animal protein was raised to 10.4% of the total ration. This required the addition of 19.5% of meat meal (53.5% protein). Carrick, Hauge and Prange (*1*) developed a ration which gave no better growth on increasing the level of meat meal (50% protein) above 19%. Holst (*4*) advocated a high protein content in rations for starting chicks and called attention to the fact that the amount of animal protein necessarily depends on the particular protein and other ingredients in the basal ration. Heuser and Norris (*3*) fed chicks on rations containing 20.9, 18.6, 16.8 and 15.2% of protein. During the first eight weeks growth ranged in order of protein content. Later, the same workers (*8*) fed rations containing 20.23, 17.93, 15.49 and 12.87% of protein, supplied by meat meal. The best growth, for the first eight weeks was obtained on the ration containing 20% protein. The paralleling of the growth curves after eight weeks with the lot receiving 15.5% protein suggested that such was the requirement for that period of growth. The efficiency of protein utilization decreased with advancing age. St. John, Carver, Halphrey, Miller and Cassell (*9*) fed rations containing 13.56, 14.43, 15.03, 16.06 and 17.17% protein, using skimmilk powder as their variable source.

[1] A contribution from the Nutritional Laboratory, Department of Poultry Husbandry. A part of this work formed the undergraduate thesis of one of the authors (J.D.M.) in fulfilment of the requirements for the degree of Bachelor of the Science of Agriculture (1932), the actual experiment being conducted in 1931.

The Ca : P ratio only varied from 2.55 : 1 to 2.13 : 1. The increase in protein levels resulted in improved growth.

It seemed evident that an investigation of the protein requirements of chicks, using a combination of protein sources was indicated. While this problem was under investigation and since its conclusion, four papers dealing with this problem have been published. Swift, Black, Voris and Funk (*10*) used a mixed protein supplement containing 75% dried buttermilk, 12.5% fish meal and 12.5% meat scrap. Their experiment was divided into four periods of four week's duration each. The rations in each of these periods varied slightly. The greatest gain for the sixteen weeks was made by the lot receiving 21.90, 21.21, 20.91 and 20.29% protein, respectively, in each of the periods. In the first four weeks the greatest gain was made by the lot receiving 22.9% protein, from four to eight weeks on 21.21%, from eight to twelve weeks on 17.31% and, from twelve to sixteen weeks on 14.74% protein. Carver, St. John, Aspinal and Flor (*2*) experimented with three levels of skimmilk powder. For the first ten weeks the most rapid growth was made on the ration containing 18% protein. Milne (*5*), using a mixed protein supplement, obtained the best growth with 23.3% protein. Morris, Thompson and Heller (*6*) obtained maximum growth with 17 to 20% crude protein, an increase to 24% giving no better growth.

EXPERIMENTAL

The experiment was conducted in the "Candee" section of the brooder house of the Poultry Department, Ontario Agricultural College. The rations were composed of the same ingredients in varying amounts. These basal mixtures were as follows:

Grain Mixture		*Protein Mixture*	
Ground Yellow Corn	100	Buttermilk Powder	100
Groung Durum Wheat	100	Fish Meal	70
Ground Barley	100		
Ground Oat Groats	100		

Mineral Mixture	
Bone Meal	2
Fine Oyster Shell	2
Fine Grit	2
Iodized Salt	1

The increasing levels of "animal protein" were added to the ration at the expense of the grain mixture, resulting in some loss of "vegetable protein", but an amount insignificant compared to the total protein of the ration, especially in view of the low biological value of this type of protein. It was obvious that the addition of protein increased the mineral content of the ration. The mineral content of all rations was equalized by decreasing the mineral supplement by an amount equal to the mineral content of the added protein, the ash content of the rations being constant at 6.3%. The Ca : P ratio varied from approximately 2.5 : 1 to 1.5 : 1 which is within the generally accepted range of 3 : 1 to 1 : 1 especially since 3% of cod liver oil was added to all rations. Green feed was supplied in the form of alfalfa leaf meal at a 4% level throughout.

The composition of the rations is given in Table. 1.

TABLE 1.—COMPOSITION OF DIETS

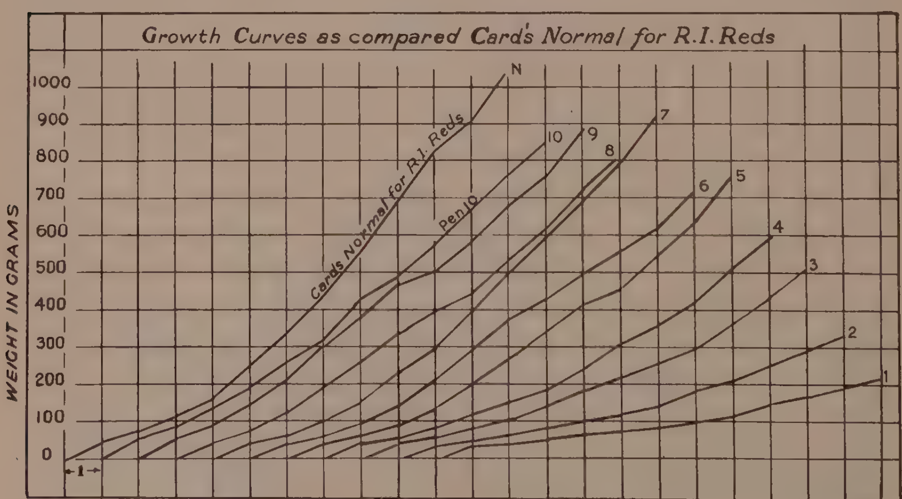
—	Pens									
	1	2	3	4	5	6	7	8	9	10
	%	%	%	%	%	%	%	%	%	%
Grain mixture	88.8	86.0	83.1	80.3	77.4	74.6	69.7	60.2	50.7	41.1
Protein mixture	0.0	3.0	6.0	9.0	12.0	15.0	20.0	30.0	40.0	50.0
Mineral mixture	4.2	4.0	3.9	3.7	3.6	3.4	3.3	2.8	2.3	1.9
Alfalfa leaf meal	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Cod liver oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
% Crude protein N = 6.25	11.6	12.7	13.7	14.4	15.3	16.3	19.0	22.7	26.4	30.0

From fifty to sixty, day-old, Barred Plymouth Rock chicks from the pedigree line of the College, hatched in a Petersime incubator, were used for each ration. The birds were weighed individually, to the nearest gram, weekly for twelve weeks. At the end of this period they were examined for sex, colour and texture of feathering, as well as the amount of feather development over the back and for head and leg development. Feed consumption was measured for three, four-week periods and a record of mortality was kept.

Influence of Protein on Growth

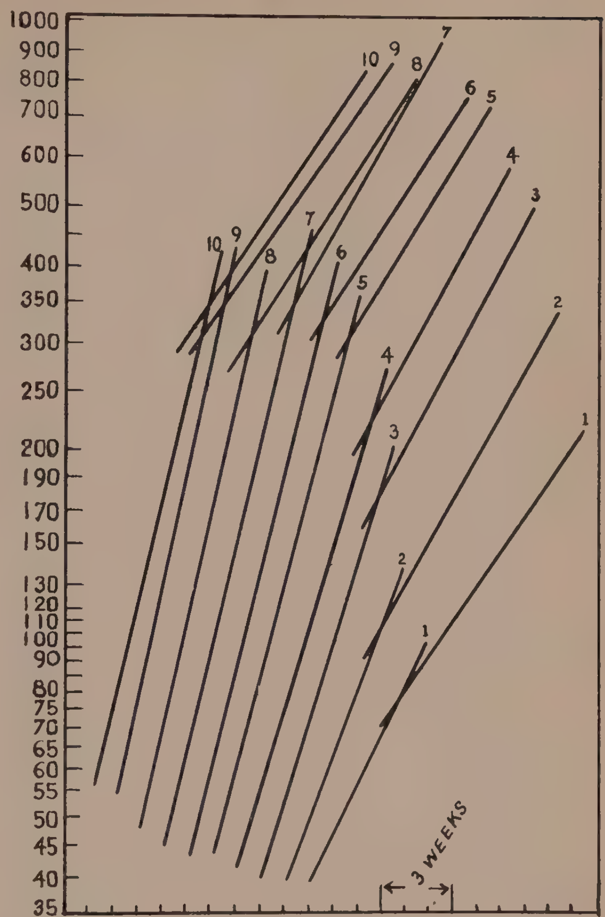
Growth curves compiled from the average weekly weights are shown in Graphs 1 and 2. The average weights of each pen by weeks is shown in Table 2.

This experiment was conducted before the importance of the vitamin B_2 complex for growth was extensively realized, together with the knowledge that commercial preparations of protein concentrates varied in their



GRAPH 1.—The influence of the protein content of the ration on the growth of chicks.

vitamin B_2 content. Although milk is an excellent source of this growth factor, it is possible that different growth responses might have been obtained if a constant source of this factor had been included in all rations. In other words, the increased growth obtained by increasing the protein mixture of buttermilk powder and fish meal, up to the optimum level, may not have been due entirely to protein *per se*, but may have been partially the result of increased vitamin B_2 in the increased amounts of the proteins, particularly the milk. However, this possibility does not invalidate the practical importance of the findings.



Graph 2. The influence of the protein content of the ration on the growth rate of chicks.

At the end of the first six weeks the greatest growth was shown by pens 8 and 9. The best growth throughout the entire period was made by pen 7. After the first six weeks pens 8, 9 and 10 grew at about the same rate. During the last two weeks pens 5, 6, 7, 8, 9 and 10 made about

Table 2. Average weights (grams) per pen per week

Age (Weeks)	1	2	3	4	5	6	7	8	9	10
1	39.3	39.4	41.0	41.0	43.6	43.6	45.1	48.0	55.1	55.7
2	46.8	50.3	56.9	57.2	65.6	61.8	64.9	75.8	87.8	84.9
3	58.1	85.8	76.6	83.4	92.4	95.3	105.1	122.8	144.5	136.4
4	70.8	86.2	108.2	120.4	137.9	143.7	156.2	191.1	209.6	190.7
5	80.6	104.5	147.6	153.6	200.9	210.2	233.5	260.8	304.2	261.1
6	89.3	121.1	188.8	188.6	270.2	295.1	294.2	336.3	383.2	338.3
7	103.1	145.7	218.1	241.5	338.5	374.3	390.1	391.7	469.1	429.1
8	120.5	188.8	260.7	315.8	414.9	427.7	494.5	440.6	500.1	487.7
9	151.1	212.6	295.1	360.6	455.6	497.1	596.2	533.2	582.0	572.5
10	170.1	253.1	364.1	422.5	542.4	566.3	693.2	615.8	679.8	667.4
11	191.4	295.1	433.1	511.7	634.3	618.2	792.6	725.9	755.5	764.2
12	218.6	335.4	513.2	592.3	759.3	723.4	925.6	808.4	879.3	847.8

the same growth rates. Pens 1 to 4 were retarded throughout the experiment. The fact that the growth of pen 10 slowly fell behind that of pens 7 and 9 shows that this ration probably contained an excess of protein, detrimental to growth, although the fact that this lot grew better than pen 8 would indicate some possible adjustment to a high protein intake.

These results indicate that during the first six weeks the protein requirement for the most rapid growth of chicks is high, being approximately 25%; from the sixth to tenth week the requirement would seem to be about 18 to 20% and after this period could probably be reduced to about 15%. The results would further indicate that the protein content of a chick mash, for use during the first ten or twelve weeks, should be about 19%. Since the birds fed on the diet containing 11.6% of protein did show some growth it is evident that the avian protein requirements for maintenance alone is less than this amount. The results are in general agreement with the conclusions of other investigators.

However, in view of other experiments to be reported later, in which excellent growth was obtained by the use of a ration containing 15.3% crude protein, it should be pointed out that the optimum protein level, using a particular type of ration as a basal, does not *a priori* indicate that such is the optimum level for all rations. Either the protein requirement is influenced by the other constituents of the diet, or, in such a ration as mentioned, a more proper blending of the proteins has been achieved. It is of interest to mention that this ration was formulated by a study of the intake of chicks given a choice of a variety of foods, that is, by cafeteria feeding. Such a ration, as well as having a lower protein content, does not contain the crude animal protein supplements, such as milk powder, fish meal or meat meal in the proportions commonly used.

It is possible, in addition, that the physical state of the ration may influence the digestibility of the ration and so effect the economy of the utilization of any individual component of the ration, such as protein. This cafeteria ration is not a finely ground mash but is coarser, due to the use of rolled or crushed rather than finely ground grains.

Efficiency of Food Utilization

The feed consumption was recorded for three four-week periods. The average food consumption per bird was calculated by dividing the total amount of feed eaten in the period by the number of birds alive in the pen at the end of the period, that is, charging the mortality against the diet.

The periodic feed consumption together with the gain per unit of feed is shown in Table 3.

During the first period the most efficient utilization of feed was made in general by the pens containing the greater amounts of protein. In the second and third periods the most efficient utilization was made in pen 7.

These results again confirm the findings that efficiency of food utilization decreases with advancing age, since in almost every case the gain per 100 grams of food eaten becomes progressively smaller.

In experiments of ths nature one factor is not taken into consideration —namely, that of allowing the birds more floor space with increase in size. A consideration of this factor might possibly make some changes in a study of food utilization.

TABLE 3.—FEED CONSUMPTION

—	Pens									
	1	2	3	4	5	6	7	8	9	10
1st 4 weeks—										
Total consumption	15890	13283	18160	12939	21338	15436	16798	26332	22700	19552
Average consumption	337.8	282.6	356.1	275.3	395.1	395.8	365.1	478.7	504.4	443.6
Consumption per 100 grams gain	482	327	329	228	286	275	233	303	241	232
Gain per 100 grams consumption	20.7	30.5	30.3	43.1	34.9	36.3	42.7	33.0	41.4	43.1
2nd 4 Weeks—										
Total consumption	13283	16798	27240	29283	45845	30074	40633	49713	42222	35185
Average consumption	332.1	399.9	555.8	681.0	898.9	812.8	944.9	937.9	981.9	902.1
Consumption per 100 grams gain	668	389	364	348	324	287	279	336	336	304
Gain per 100 grams consumed	14.6	25.6	27.4	28.6	30.8	34.8	35.8	29.4	29.7	32.8
3rd 4 Weeks—										
Total consumption	20657	32915	39044	41768	60382	44080	64595	54615	58112	70597
Average consumption	573.8	843.9	1055.2	1070.9	1312.6	1296.4	1584.0	1188.1	1417.3	2073.7
Consumption per 100 grams gain	584	575	417	387	316	438	298	323	373	575
Gain per 100 grams consumed	17.1	17.3	23.9	25.8	31.6	20.5	33.4	30.9	29.4	17.3

Influence of Protein on Mortality

The weekly mortality together with the per cent mortality for each pen is shown in Table 4.

TABLE 4.—MORTALITY

—	Weeks												Percent mortality
	1	2	3	4	5	6	7	8	9	10	11	12	
Ration 1	0	5	5	0	0	2	4	1	2	1	0	1	38.6
2	3	2	5	3	1	1	2	1	1	0	2	0	30.0
3	2	3	0	1	0	0	1	1	1	4	5	2	31.7
4	3	5	3	1	1	0	1	2	1	1	2	0	28.9
5	1	2	0	1	0	1	0	2	1	1	0	3	17.2
6	6	8	2	0	0	1	0	1	0	1	1	1	27.9
7	4	4	1	0	1	0	1	1	1	0	0	1	18.1
8	3	0	2	0	0	2	0	0	3	2	1	1	18.3
9	0	3	0	0	0	1	0	1	1	0	0	1	14.5
10	3	4	1	1	0	0	3	2	2	1	1	1	30.1

In calculating the per cent mortality the first week's mortality was disregarded, in order to avoid, in so far as possible, any death not strictly due to a dietary factor. However, some diets, used in experimental work in this laboratory have shown 100% mortality during the first week, indicating that mortality may be influenced by diet, in severe cases at least, during the first week.

The general trend of mortality indicates that the nearer the protein level approaches the optimum, other factors being equal, the lower the mortality. An excess protein content, as would be expected, raises the death rate.

FIGURE 1. A "blue" cockerel showing the effect on the barred feather pattern of excessive protein. The males become lighter in colour as shown in Figures 1 to 3 inclusive. A similar condition results on low protein rations.

FIGURE 3. A "white" cockerel fed on the high-protein rations.

FIGURE 5. A "smutty" pullet, showing the indistinct character of the barred pattern on high protein rations.

FIGURE 2. A "grey" cockerel, showing the effect of excessive protein in the ration on the barred feather pattern.

FIGURE 4. A "dark" pullet, showing the darkening of the barred feather pattern due to high protein rations.

FIGURE 6. A "blue" pullet fed on a high protein ration. Note, not only the upset in the barred feather pattern, but also the "square" feather tips.

Influence of Proteins on Feathering

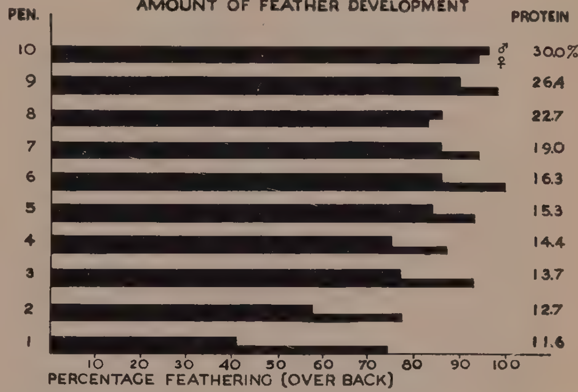
1. Amount of Feathering

The percentage of feathering over the back of each bird was estimated. Averages were calculated for the males and females in each group. These are shown in Chart 1.

As will be seen there is a direct relationship between the amount of protein in the ration and the amount of feathering of the cockerels, the amount of feathering increasing with additional protein. In the case of the pullets this relationship is not so marked, although the birds fed on the lower protein levels are definitely retarded in feather development. On almost all diets the pullets showed more feather development than did the corresponding cockerels.

CHART I

INFLUENCE OF PROTEIN ON AMOUNT OF FEATHER DEVELOPMENT



2. Colour and Barring of Feathers.

During the seventh to eighth week there was a tendency towards the "destruction" of the barred feather pattern in the plumage of the birds on the low and high protein diets. This may be an actual destruction, or an inhibition of, or some metabolic upset, disturbing the deposition of pigment. The results of this "destruction" was a change in colour of the feathers. The cockerels became bluish grey, blue and in a few cases almost white in colour. No explanation can be offered at the present time for this phenomenon. A few typical birds taken from various pens showing some of the colour variations are shown in the photographs.

The development of the barring was estimated for both sexes. These results are shown in Table 5.

TABLE 5.—INFLUENCE OF PROTEIN ON FEATHERING

Pen	Total feathering		Percentage barring	
	Males	Females	Males	Females
	%	%	%	%
1	41	74	46	83
2	58	77	83	85
3	77	93	54	96
4	75	87	91	94
5	84	93	92	100
6	86	100	90	60
7	86	94	81	66
8	86	83	50	60
9	90	98	94	45
10	96	94	56	44

It will be seen that the development of barring in the feathers of cockerels is due primarily to some factor other than dietary. In the case of pullets the development of barring would seem to definitely be associated with the diet. These results also suggest that the optimal protein requirement for growth is not the same as that for the development of the proper barring in pullets. A high protein content definitely upsets

this feather development. This aspect of the problem is of particular importance to breeders of exhibition birds, in which a proper blend must be made between growth and feather barring. It would seem from these results that this blend would not be easily obtained. Any change in colour of growing birds would indicate that the protein content of the ration was incorrect.

3. Texture and Contour of Feathers

In some cases the feather web approached the silky appearance of the feather fluff found on the lower shaft, giving the birds the so-called "silky appearance". Again this abnormality in feather development did not seem in cockerels to be definitely associated with the diet, although there is some relationship to both high and low protein levels. With pullet feathering this abnormality definitely developed on the two high protein rations, in pens 9 and 10 and on the low protein intake in pens 1 and 2.

In connection with feather development, it might be of interest to point out that some birds on a high protein intake, grew feathers whose shape or contour, as well as texture, resembled the feathering of squabs, that is, having square ends. This type of feathering has also been developed on a ration containing a high content of ground peas (see Figure 7).

FIGURE 7. Showing the typical "square" feather development on a ration with a high content of peas.

These dietary influences on feather development would not be so noticeable and in some cases would not occur if white birds were used. Most workers in this field have used White Leghorn chicks which would account for their failure to note these abnormalities.

The Influence of Protein on the Development of Slipped Tendons

Many investigators have associated the development of slipped tendons with the protein content of the ration, in most cases with an excess of this nutrient. Some have indicated that this condition is associated with the protein *per se*, others with an excess or unbalancing of the mineral content of the diet, caused by the excess protein. In this experiment the ash content of the ration was the same in all diets, which should prevent in so far as possible any excess or unbalancing of minerals, especially in view of the fact that the Ca : P ratio varied within normal limits.

During the fourth week one bird in Pen 3 and one in Pen 2 during the eighth week, developed slipped tendons. These were the only cases

on a low protein intake. In Pen 10 the first case of slipped tendons appeared at the end of the third week, two more developed at the end of the fifth week, one during the sixth week and two during the seventh week, making a total of six in all. The birds in this pen were the only ones of these receiving a high protein diet which showed this abnormality.

These results would indicate, in view of the few birds which developed slipped tendons, that this abnormality is associated with some factor other than the protein content of the diet. However, since it is in general the most rapidly growing birds, on any ration which seem predisposed to develop slipped tendons, the protein in a ration may be a contributary factor. It may be that the growing chick gains in weight so rapidly on a high protein intake, that muscular development advances ahead of bone formation, and hence the bones are unable to support the chick, the consequent bending of the bones allowing the tendons to slip to either side.

Influence of Protein on Head Development

During this experiment notes were made on the development and formation of the birds' heads, since "type of head" is a factor in estimating the constitution of the bird in relation primarily to vigour and egg production. Most of the birds receiving a low protein diet developed the condition known as "crow-head", characterized by a narrow skull and a long narrow beak as may be seen in Figure 8.

FIGURE 8. Showing a bird with a "crow head", a result of an inadequate protein intake.

The relationship between this crow head development was very definitely associated with inadequate protein intake, as will be seen in Table 6.

TABLE 6.—INFLUENCE OF PROTEIN ON HEAD DEVELOPMENT

Pen	Percent Crow Heads	
	Males	Females
	%	%
1	87	83
2	50	70
3	24	27
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

Many poultrymen believe that a genetic factor is responsible for the production of this head type. These results would show that it is due to a dietary rather than a hereditary factor. It would seem probable that any ration which is deficient in any factor necessary for the proper nutrition of chicks would result in the production of "crow heads".

SUMMARY

1. The optimum protein content for growth in chicks during the first six weeks on the usual type of ration is approximately 25%, from six to ten weeks 18 to 20%, and after this time the protein requirement is about 15%.

2. The optimum amount of protein in a diet for growing chicks over a twelve week period is approximately 19%.

3. A level of protein over 25% is approaching an excess, detrimental to growth.

4. The efficiency of food utilization decreases with age, and mortality is lessened, other factors being equal, the nearer the protein content to optimum.

5. Both high and low protein intake, particularly the former, tend to destroy the barring and to change the contour and texture of feathers. The optimum protein level for growth is not necessarily the optimum level for correct feather development.

6. Slipped tendons are not due primarily to a high protein intake *per se.*

7. The development of "crow heads" is a dietary rather than a genetic factor.

ACKNOWLEDGMENTS

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Since the conclusion of this experiment Gerricke and Platt (New Jersey Agric. Exp. Station, Bulletin No. 543, 1932), have found that feather development in Barred Rock chicks was improved proportionally with increased amounts of protein in the ration.

REFERENCES

1. Carrick, C. W., Hauge, S. M., and Prange, R. W. A milkless, greenless, gritless, all-mash ration for growing chicks. Poul. Sci. *6* : 162. 1927.
2. Carver, J. S., St. John, J. L., Aspinall, F. E., and Flor, I. H. Protein requirements of chickens. Poul. Sci. *11* : 45. 1932.
3. Heuser, G. F. and Norris, L. C. The influence of the protein level on the rate of growth in chickens. Report World's Poultry Congress, 328. 1930.
4. Holst, W. F. A consideration of the protein requirements of growing birds. Report World's Poultry Congress, 183. 1927.
5. Milne, H. L. Protein requirements of growing birds. Sci. Agric. *12* : 604. 1932.
6. Morris, L., Thompson, R. B., and Heller, V. G. The effect of varying the amounts of protein in the poultry ration on chick growth and subsequent egg production. Poul. Sci. *11* : 364. 1932.
7. Mussehl, F. E., and Gish, D. E. The protein requirements of growing chicks. Poul. Sci. *5* : 20. 1925.
8. Norris, L. C. and Hueser, G. F. The relation of the protein requirement of chicks to the rate of growth. Poul. Sci. *9* : 378. 1930.
9. St. John, J. L., Carver, J. S., Helphrey, J. P., Miller W. and Cassel, L. W. The effect on growth of various protein levels of dry skimmilk in a chick mash. Poul. Sci. *9* : 320. 1930.
10. Swift, R. W., Black, W., Voris, L., and Funk, E. M. The optimum protein content of rations for growing chicks. Poul Sci. *10* : 288. 1931.

Résumé

ÉTUDE SUR LA QUANTITÉ DE PROTÉINE NÉCESSAIRE AUX POUSSINS. J. D. McConachie, W. R. Graham, Jr., et H. D. Branion, Collège d'Agriculture de l'Ontario, Guelph, Ont.

La quantité optimum de protéine pour la croissance des poussins qui reçoivent une ration du type habituel est d'environ 25% pendant les six premières semaines; elle est de 18 à 20% de six à dix semaines, après quoi elle est d'environ 15%. Pour une période de douze semaines la quantité optimum de protéine que doit contenir le régime alimentaire des poussins est d'environ 19%. Un niveau de protéine dépassant 25% est excessif et peut nuire à la croissance. L'utilisation efficace de la nourriture décroît avec l'âge, et la mortalité est plus faible lorsque la proportion de protéine se rapproche de l'optimum, tous les autres facteurs étant égaux. Une absorption élevée et faible de protéine, spécialement la première, tend à détruire les barres et à changer le contour et la texture des plumes. Le niveau optimum de protéine pour la croissance n'est pas nécessairement le niveau optimum pour le bon développement des plumes. La haute absorption de protéine n'est pas la première cause du glissement des tendons. Le développement de "têtes de corneille" est un facteur diététique plutôt qu'un facteur génétique.

BACTERIOLOGICAL STUDIES OF DRESSED POULTRY

I. PRELIMINARY INVESTIGATIONS OF BACTERIAL ACTION AT CHILL TEMPERATURES[1]

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INTRODUCTION

In March, 1934, the need for bacteriological study of the changes occurring in chilled dressed poultry was suggested by Mr. W. A. Brown, Chief, Poultry Services, Dominion Live Stock Branch. While it was realized that microbial spoilage occurred at the temperatures commonly employed for storing the chilled product, it was felt that more definite information was required concerning the nature of the bacterial changes involved and the factors which determine the keeping quality of dressed poultry. Bacteriological studies were undertaken extending from March, 1934, to January, 1935, in the course of a co-operative investigation by the Department of Agriculture and the National Research Council in which chemical and physical aspects of the problem were also considered.

Investigational work on the microbiology of animal products in cold storage has been largely confined to the study of meat. While many of the findings are doubtless applicable to the question of refrigeration of poultry, yet differences in the physical nature of the products involve the consideration of special factors influencing keeping quality. In 1908 Stiles (*8*) reporting the examination of chickens kept in cold storage at a temperature from 2° to 8° F. found living organisms in the flesh after 610 days. In a study of market cold-storage chickens held at 13° F. Pennington (*5*) found appreciable numbers of organisms in the edible portions even after 4 years, though the numbers tended to decline with storage.

As the temperatures of storage employed by the above-mentioned workers are below those at which microbial growth may be said to occur, deterioration of poultry under such conditions results from physico-chemical and enzymatic changes rather than from microbiological action. The lowest temperature recorded for bacterial growth appears to be −7.5°C (18.5° F.) as reported by Bedford (*1*) in a study of marine organisms. At this temperature certain species of *Micrococcus* and *Achromobacter* were capable of slow development. In a study of the cold tolerant microflora of meat, Haines (*3*) found that with the mold *Sporotrichum carnis* growth did not cease until between −7° and −10° C. (14° to 19.4° F.). Below −10° C. (14° F.), according to the same author (*4*), no microbial growth of any kind occurs on frozen meat. Berry (*2*) places the critical temperature for microbial growth at 15° to 20° F.

At temperatures approximating 0° C. (32° F.), however, various species of bacteria, molds and yeasts are capable of development, as has been shown by numerous studies with organisms isolated from soil, fish, meat, vegetables and other sources. Consequently microbial development might be expected to be an important factor in determining the keeping quality

[1] Contribution No. 34 (Journal Series) from the Division of Bacteriology, Dominion Experimental Farm, Ottawa.

[2] Dominion Agricultural Bacteriologist and Graduate Assistant respectively.

of poultry stored at such temperatures commonly employed for the chilled, as distinct from the frozen, product. In a study of the effect of different holding temperatures upon various chemical and biological changes, Pennington, Hepburn, St. John and Witman (7) noted increases in bacteria in both muscles and skin of chickens held at 0° C. and 7.2 to 12.8° C. though the increases were much less marked than at ordinary room temperatures.

EXPERIMENTAL

Preliminary Tests

The poultry placed at our disposal was supplied by the Live Stock Branch, Dominion Department of Agriculture and the Poultry Division Experimental Farms. Considerable preliminary work was conducted on various lots of packed birds held at 30° and 32°F. with the object of testing various methods of analysis and the elaboration of a technique most suitable for the evaluation of changes occurring during storage.

Bacterial plates of the numbers of organisms on the skin surface and in the muscular tissue, using incubation temperatures of 37°, 28°, 20° and 2.2° C. showed that 37° did not permit of growth of the maximum numbers of organisms, while incubation at 2.2° or 20° gave no advantage over 28° C. Of the organisms which developed, both from the skin and muscle samples, a large proportion were ammonifying bacteria. *Bact. coli* was present in only very insignificant numbers, indicating no development of this type either on the surface or in the tissue. Growth of organisms on the skin was more definite than in the tissues. Counts from the thigh showed greater variations and were generally higher than from the breast muscle. Tests for anaerobes gave no more pronounced indication of growth than aerobic plates in the birds examined.

STUDY OF DRESSED POULTRY STORED AT 30° AND 32° F.

In this experiment 144 birds were examined, which had been prepared by the Poultry Division, Experimental Farm and the Poultry Services Division, Live Stock Branch. After killing and dressing, the birds were transported to the refrigeration plant of the National Research Council and pre-cooled to 30° F. over night. They were then packed in the regular manner, 8 birds to a box. Two boxes (16 birds) were examined when freshly packed and the remainder stored in equal lots at 30° F. and 32°F. respectively. At the end of 2, 4, 6 and 8 weeks two boxes (16 birds) were removed from each of the cold storage chambers for bacteriological examination. To facilitate handling the birds, the killing, packing, etc. were so arranged that the analytical work at every sampling was spread over four consecutive days.

Methods

Bacterial counts were made of the skin and the breast tissue of each bird. For the former, four portions of skin, 1 sq. cm. each, were cut with a scalpel from the breast, back, rump and thigh respectively and added to 100 cc. sterile 0.85% NaCl in a 200 cc. Erlenmeyer flask containing approximately 15 gm. broken glass. The flask was shaken for 5 minutes and further dilutions prepared as required. Duplicate plates were prepared from each dilution employed, using nutrient agar and incubating at 28 °C. for 4 days. Bacterial numbers were estimated per square centimeter skin surface.

For the examination of the breast tissue, the outer breast muscle from one side was first cut away and 4.0 gm. of the inner muscle tissue removed aseptically and weighed into a sterile 200 cc. Erlenmeyer flask containing 15 gm. broken glass. After the addition of 100 cc. sterile saline the flask was shaken for 10 minutes. Nutrient agar plates were prepared, incubated at 28° C. for 4 days and numbers of bacteria estimated per gram of tissue.

RESULTS AND DISCUSSIONS

The results from the bacteriological analyses of 144 birds are shown in Table 1 in which are summarized the logarithmic averages for the counts obtained from 16 birds in each temperature and storage period group. It will be noted from the table that during the period of storage there is a notable increase in the average bacterial numbers on the skin surface at both temperatures, the increase being more pronounced at 32° than at 30° F.

It was found that the birds acquired a surface odour before there was any apparent decomposition of, or significant increase of bacteria in, the muscle tissue examined. In addition to the pronounced increase of bacteria on the skin during storage it was observed that the odour from the plate cultures was very similar to that which eventually developed on the stored birds. It is therefore believed that the deterioration of dressed poultry at 30° and 32° F. to the point where the birds acquire a noticeable odour is essentially a surface spoilage due to bacterial growth. With the birds held at 32° F. this initial sign of spoilage was first observed after 4 weeks, being apparent with those stored at 30° F. at the following (6 wk.) sampling. From Figure 1, in which the logarithms of the average bacterial counts are plotted against time, it is noted that at approximately log. 6.4 (2,500,000 per sq. cm.) the stage is reached at which surface odour is first apparent. From the data there is indication that birds held at 30° F. remain approximately one week longer than at 32° F. before reaching the initial stage of spoilage, namely 5 weeks as compared with 4.

Compared with the bacterial counts of the skin, those obtained from the examination of the breast tissue were very low. The presence of small numbers in the first examination (1 day) confirms the observations of Pennington (*6*), who showed that the presence of a few bacteria twenty-four hours after death, even with careful and rapid chilling, could be demonstrated in breast and thigh muscle. During

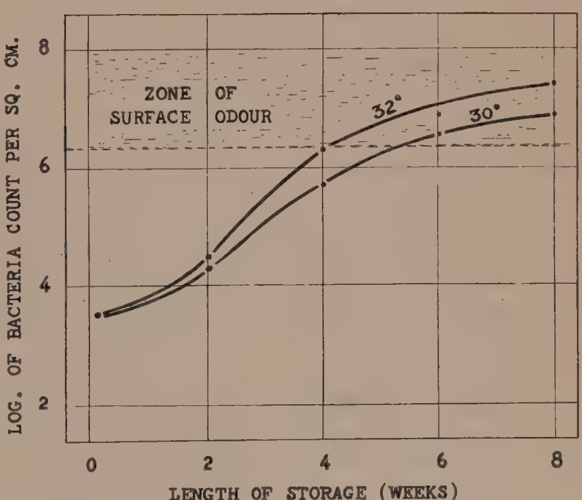

FIGURE 1. Relation of length of storage at 30° and 32° F. to development of bacteria on skin of dressed poultry.

TABLE 1.—SUMMARY OF BACTERIAL COUNTS FROM SKIN SURFACE AND INNER BREAST MUSCLE OF 144 BIRDS

Storage period	Stored at 30° F.			Stored at 32° F.		
	No. of birds	Bacterial counts (logarith, aver.)		No. of birds	Bacterial counts (logarith. aver.)	
		Skin (sq. cm.)	Muscle (gm.)		Skin (sq. cm.)	muscle (gm.)
1 day (precooled)	16	3,380	32	—	—	—
2 weeks	16	20,400	27	16	30,800	32
4 weeks	16	551,800	121	16	2,021,000	376
6 weeks	16	3,635,000	776	16	7,396,000	1,880
8 weeks	16	7,920,000	672	16	25,300,000	290

TABLE 2.—CHARACTERISTICS OF PREDOMINANT BACTERIAL TYPES ON SKIN OF DRESSED POULTRY STORED AT 30° AND 32° F.

Cult. No.	Form	Size (μ) N.A. 20°	Motility	Spores	Gram stain	Gelatine liquef.	NO_3 reduction	Acid (dextrose)	Gas (dextrose)	Indol	Action in milk	Optimum temp. °C.	Growth at 37° C. (10 days)	Chromogenesis	Generic classification
1	coccus	0.7–1.1 diam.	–	–	–	–	+	–	–	–	–	20–28	–	–	Micrococcus
2	coccus	0.6–0.9 diam.	–	–	–	–	+	–	–	–	—	20–28	—	—	Micrococcus
3	rod	1.2–3.5 × 0.5–0.6	+	–	–	+	–	+	–	–	peptonized	20	sl.	—	Achromobacter
4	rod	0.8–2.0 × 0.5–0.7	–	–	–	+	–	+	–	–	—	20	—	—	Achromobacter
5	rod	0.8–1.8 × 0.6	+	–	–	–	–	+	–	–	—	20–28	—	yellow	Flavobacterium
6	rod	0.8–2.0 × 0.5–0.7	–	–	–	–	–	+	–	–	—	20	—	—	Achromobacter

the period of our test, increases were relatively slight and irregular, the numbers of organisms being regarded as too low to indicate microbial decomposition. It was furthermore noted that no spoilage of tissue was present which could be determined organoleptically. On the other hand surface deterioration progressed until the conclusion of the tests. To the characteristic surface odour due to bacteria was added at later stages the distinctive musty odour arising from the development of molds, more particularly on the birds stored at 32° F.

STUDY OF BACTERIAL TYPES

In addition to the quantitative determinations reported above, a study was made of the bacterial types most prevalent on the skin of the birds. For this purpose the plate cultures made at the end of the period of storage were used, by which time any possible adjustment to a typical cold storage microflora would have taken place.

From the plate cultures from 8 different birds at 30° F. and at 32° F. isolations were made from the predominant types of colonies, at least four colonies from each plate. In this way some 70 cultures were obtained which, after morphological and physiological comparisons, were reduced to six types regarded as distinct species. The comparatively small number of types predominating confirmed the macroscopic observations of colony types on the plate cultures. A summary of the characteristics of the six predominant types is given in Table 2.

With respect to the types occurring at the different storage temperatures, no special distinction was possible between the birds stored at 30° and 32° F. This tends to confirm observations made throughout the experiments in which the plate cultures were similar in general colony appearance, odour, etc. The effect of the difference of temperature studied on the bacterial development was thus quantitative rather than qualitative. It is of interest to note that the predominant types were species which though capable of growth at temperatures near the freezing point, develop best at 20° to 28° C. Little or no growth occurred at 37° C., supporting earlier observations as to the unsuitability of this temperature for studying the microflora of chilled poultry.

SUMMARY

The deterioration of dressed poultry at 30° and 32° F. to the point where the birds acquire a noticeable odour is essentially a surface spoilage.

The first odour noticeable is due to the development of bacteria on the skin surface, and reaches an extent which may be objectionable before there is any significant decomposition of, or notable increase of bacteria in, the muscular tissue. The first signs of surface odour are apparent when the bacteria count on the skin has exceeded approximately 2,500,000 per square centimetre.

From the bacteriological point of view birds held at 30° F. remain approximately one week longer before reaching the initial stage of spoilage than those held at 32° F.

The predominant types of bacteria developing on the skin during storage were representative of the genera *Micrococcus*, *Flavobacterium* and *Achromobacter*, six species being described, all cold-tolerant rather than

cold-loving. Differences in bacterial development at 30° and 32° F. were quantitative rather than qualitative.

REFERENCES

1. Bedford, R. H. Marine bacteria of the Northern Pacific Ocean. The temperature range of growth. Contrib. to Can. Biol. and Fish. 7, No. 34 (Series C. No. 10) 1933.

2. Berry, J. A. Cold-tolerant micro-organisms and frozen pack. The Canner. Feb. 24, 1934.

3. Haines, R. B. The influence of temperature on the rate of growth of *Sporotrichum carnis*. J. Exp. Biol. *8* : 379. 1931.

4. ——————. Report of the Food Invest. Board for 1931. Sect. A. Meat. p. 46-51. 1932.

5. Pennington, M. E. A preliminary study of the effects of cold storage on eggs, quail and chickens (H. W. Wiley). U.S. Dept. Agr. Bur. Chem. Bull. No. 115, Sec. 4, p. 57-99. 1908.

6. ——————. Proc. First Int. Cong. Refrig. Ind. *2* : 243. 1909.

7. ——————, Hepburn, J. S., St. John, E. Q. and Witmer, E. The influence of temperatures above freezing on the changes in chemical composition, bacterial content and histological structure of the flesh of the common fowl. Proc. Amer. Soc. Biol. Chem. 1916. J. Biol. Chem. *29* : xxxi-xxxii. 1917.

8. Stiles, G. W. A preliminary study of the effects of cold storage on eggs, quail and chickens. (H. W. Wiley). U.S. Dept. Agr. Bur. Chem. Bull. No. 115. Sec. 3, p. 44-57. 1908.

Résumé

ÉTUDES BACTÉRIOLOGIQUES DES VOLAILLES HABILLÉES. A. G. Lochhead et G. B. Landerkin, Ferme expérimentale fédérale, Ottawa.

La détérioration à une température de 30° et 32° F. des volailles habillées jusqu'au point où l'odeur devient prononcée, est essentiellement une décomposition de surface. La première odeur constatée est due au développement de bactéries sur la surface de la peau; elle devient assez forte pour incommoder avant qu'il y ait une décomposition significative du tissu musculaire ou une augmentation notable de bactéries dans ce tissu. Les premiers signes d'odeur de surface sont apparents lorsque la numération des bactéries sur la peau dépasse environ 2,500,000 par centimètre carré. Au point de vue bactériologique, les sujets conservés à 30° F. mettent environ une semaine de plus à atteindre les premières phases de la décomposition que ceux qui sont conservés à 32° F. Les types prédominants de bactéries qui se développent sur la peau pendant l'entreposage représentaient les genres *Micrococcus*, *Flavobacterium* et *Achromobacter;* six espèces sont décrites, toutes tolérantes au froid plutôt que recherchant le froid. Les différences notées dans le développement des bactéries à 30° et 32° F. étaient des différences quantitatives plutôt que qualitatives.

THE EFFECT OF FEEDING DEAMINIZED VS. UNTREATED COD LIVER OILS UPON GROWTH, EGG PRODUCTION AND MORTALITY OF POULTRY[1]

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Cod liver oils of high free fatty acid content have been discriminated against for feeding purposes for some time past. Investigators have been unable to show, however, that free fatty acids similar or identical to those commonly found in cod liver oils are in any way deleterious when fed to poultry. Oils of high content have been shown to cause poor growth, lack of uniformity and high mortality in growing chicks, but this effect has not been shown to be caused by the fatty acids of the oil *per se*, since no attempt was made to show that this fraction was solely responsible. A review of the literature indicated that cod liver oils were not made up entirely of neutral glycerides but contained impurities mostly of a nitrogenous nature, originating from the livers from which the oil was rendered. Among these, certain amines (products of protein decomposition) were sometimes found which were decidedly toxic in their biological effect to mice and, when in pure form, to small birds. In view of these facts a series of cod liver oils was examined for content of this nitrogenous fraction and a cod liver oil of medium quality tested biologically upon poultry for possible toxic effect.

Nitrogen determinations upon a series of oils indicated the presence of such a fraction in even carefully refined oils for human consumption. Although not necessarily always the case, the analyses indicated that, usually, high free fatty acidity and high nitrogen content were to be found in the same oils. Oils which were prepared by the sun rendering process were higher in the nitrogenous fraction than those steam rendered. A very representative composite sample of pilchard oil (a body oil) was remarkably low in this fraction. A cod liver oil known to have been made from stale livers was high in free fatty acids and nitrogen even though steam rendered. It appeared therefore, that oils originating from stale livers and/or improperly processed were apt to be high in both fractions.

A representative sample of a sun rendered cod liver oil containing 29.6% of free fatty acids (expressed as oleic) and 0.31% of nitrogen was used for experimental purposes. This oil was medium dark in colour and not particularly strong or fishy in odour. In these respects it might be said to be a sun rendered oil of better than average quality. By the use of a suitable technique, the nitrogenous fraction was removed from this oil without interfering with its vitamin potency as indicated by biological tests. This oil, thus deaminized, was fed to large groups of chicks through the growing, rearing and first year egg production periods as 1% of a ration previously proven to be satisfactory for the purpose required (growth and egg production). This treatment was controlled by feeding the same oil in its original condition without deaminization or treatment of any kind to comparable birds.

The following results were obtained. The feeding of the oil containing the nitrogenous impurities reduced the growth of the chicks during the brooding and rearing periods. The effect was much more marked with the cockerels than the pullets. It also greatly increased the variability of the chicks to which it was fed. The removal of this nitrogenous fraction also permitted the pullets receiving the oil, thus treated, to make more efficient use of their feed for purposes of egg production. Mortality was slightly in favour of the individuals receiving the deaminized oil in every case.

[1] Author's abstract of a material which will appear shortly as a technical bulletin of the Dominion Department of Agriculture, Ottawa.

[2] Poultry Husbandman.

It is concluded that oils containing appreciable quantities of nitrogenous products should not be used for feeding purposes. Since in most cases these oils will also be high in free fatty acids, oils of high free fatty acid content should be discriminated against. Sun rendered oils, or oils not so rendered, but arising from stale livers, will usually be high in both free fatty acid and nitrogen content and should not be used for feeding purposes. It would appear from the results of this investigation that oils of high free fatty acid content shown previously by other investigators to cause poor growth with marked lack of uniformity and very high mortality were probably also high in this nitrogenous fraction and that this fraction was responsible for the results obtained. Since the oils which they used were purchased upon the open market (1930), the availability of such oils is evident. It may be appreciated that very serious consequences may follow their use.

Résumé

L'EFFET DE L'EMPLOI D'HUILE DE FOIE DE MORUE PRIVÉE ET NON PRIVÉE DE SES VITAMINES SUR LA CROISSANCE, LA PONTE ET LA MORTALITÉ DES VOLAILLES. H. S. Gutteridge, ferme expérimentale centrale, Ottawa.

L'auteur conclut que les huiles qui renferment une quantité appréciable de produits azotés ne devraient pas être employés pour l'alimentation. Comme la plupart des huiles de ce genre ont une forte teneur en acides graisseux libres, la présence d'une grosse quantité d'acides graisseux libres indique que ces huiles ne devraient pas être employées. Les huiles extraites au soleil ou les huiles non extraites de cette façon mais provenant de foies rances sont généralement riches en acides graisseux libres et en azote et ne devraient pas être employées pour l'alimentation. Il semble d'après les résultats de cette enquête, que les huiles qui contiennent une forte proportion d'acides graisseux libres et qui ont été la cause d'une pauvre croissance, d'un manque prononcé d'uniformité et d'une mortalité très élevée dans les expériences faites par d'autres investigateurs, contenaient aussi probablement une forte proportion de cette fraction azotée et c'est cette fraction qui a été la cause des résultats obtenus. Comme les huiles dont ils se sont servis avaient été achetées sur le marché public (1930), on voit que l'emploi de ces huiles peut être suivi de conséquences très sérieuses.

THE ECONOMIC SITUATION

PREPARED IN THE AGRICULTURAL ECONOMICS BRANCH, DEPARTMENT OF AGRICULTURE, OTTAWA, LARGELY FROM BASIC DATA COLLECTED BY THE DOMINION BUREAU OF STATISTICS

The index number of wholesale prices in Canada was slightly lower in May. Lower prices for wheat and silver were the chief factors in the recession. The index of prices of vegetable products fell from 69.4 in April to 68.0 in May. Prices of non-metallic minerals and of chemicals and allied products were also lower. The most substantial advance was registered in the non-ferrous metals group. This index rose from 67.9 to 70.7. On the whole, however, prices were lower and the index of wholesale prices declined to 72.3.

Retail prices.—The index of retain prices and costs of services was unchanged in May. Prices of foods were slightly higher and the rental index computed only in October and May was again higher. Fuel prices showed not only a season of decline but prices were below those of a year ago. Retail prices in May, 1935, were in general only a little higher than in May, 1934.

Physical Volume of Business.—The physical volume of business reached a new high point in the period of recovery, being 103.2 compared with 100 in 1926 and 98.3 in April of this year. The index of industrial production advanced from 97.7 to 104.4. Mineral production was lower than in April although exports of nickel, shipments of silver, imports of bauxite and coal production showed substantial gains. The index of manufacturing advanced from 94.0 to 105.1. Practically all the industries included in this group showed increased activity but iron and steel production was slightly lower than in the previous month and automobile production dropped from 102.9 to 87. The index of construction was 38.1 compared with 37.1. The number of contracts awarded was higher but building permits were lower and the cost of construction remained stationary. Car loadings were somewhat lower. Exports and imports were both higher. The agricultural factors will be discussed in detail in another section. It may, however, be pointed out that agricultural marketings and cold storage holdings were lower in May than in April.

Agricultural Products.—The index number of wholesale prices of Canadian farm products receded to 64.1 in May. There was a very pronounced advance in the prices of animal products, the index rising from 72.9 in April to 74.4 in May. Prices of hogs and lambs advanced sharply because of comparatively light shipments. Prices of good to choice steers were higher both at Toronto and Winnipeg during the first half of the month but declined later on because of weaker prices in United States. Prices of calves were lower in both Toronto and Winnipeg. The index of prices of field products declined from 59.8 in April to 58.0 in May. The average price of No. 1 Manitoba northern wheat was nearly two cents below that during April, while prices of oats dropped a little less. The average price of No. 2 C. W. barley declined from 45.9 to 42.3 Cents. No. 1 C. W. Flax dropped from 140.9 to 134.0. The wheat crop in Western Canada is more satisfactory than a year ago. Southern Saskatchewan is reported to have a very fair prospect and while it has been very dry in sections of both Alberta and Saskatchewan, there is prospect of at least an increase in supplies of feed. In sections, however, the crop is late and favourable harvest weather will be necessary. Stem rust is reported to be prevalent.

The index of grain marketings declined from 91.8 in April to 86.3 in May. There was a smaller movement in wheat, oats, barley, rye and flax. The index of shipments of wheat fell more than seven points, that for oats nearly nine points, and the index for barley marketings was about three points less than that registered in April.

Total live stock marketings were only slightly lower than in April and the index receded less than two points. The most marked decline was in the movement of sheep and lambs. This index is based on deliveries at public markets.

ANNUAL AND MONTHLY INDEX NUMBERS OF PRICES AND PRODUCTION COMPUTED BY DOMINION BUREAU OF STATISTICS

Year	Wholesale Prices 1926 = 100				Retail prices and cost of services (5)	Production (6) 1926 = 100			
	All commodities (1)	Farm products (2)	Field products (3)	Animal products (4)		Physical volume of business	Industrial production	Agricultural marketings	Cold Storage holdings
1913	64.0	62.6	56.4	77.0	65.4				
1914	65.5	69.2	64.9	79.0	66.0				
1915	70.4	77.7	76.9	79.2	67.3				
1916	84.3	89.7	88.4	92.3	72.5				
1917	114.3	130.0	134.3	119.6	85.6				
1918	127.4	132.9	132.0	134.7	97.4				
1919	134.0	145.5	142.4	152.5	107.2	71.3	65.5	48.1	47.1
1920	155.9	161.6	166.5	149.9	124.2	75.0	69.9	52.6	94.2
1921	110.0	102.8	100.3	108.5	109.2	66.5	60.4	65.2	86.4
1922	97.3	86.7	81.3	99.1	100.0	79.1	76.9	82.6	82.8
1923	98.0	79.8	73.3	95.1	100.0	85.5	83.8	91.4	87.6
1924	99.4	87.0	82.6	97.2	98.0	84.6	82.4	102.5	114.9
1925	102.6	100.4	98.1	105.7	99.3	90.9	89.7	97.2	108.6
1926	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1927	97.7	102.1	99.9	105.7	98.4	106.1	105.6	103.6	110.0
1928	96.4	100.7	92.6	114.3	98.9	117.3	117.8	146.7	112.8
1929	95.6	100.8	93.8	112.5	99.9	125.5	127.4	101.1	109.6
1930	86.6	82.3	70.0	102.9	99.2	109.5	108.0	103.0	128.4
1931	72.2	56.3	43.6	77.6	89.6	93.5	90.4	99.0	125.7
1932	66.7	48.4	41.1	60.7	81.4	78.7	74.0	114.3	120.1
1933	67.1	51.0	45.8	59.6	77.7	79.7	76.8	105.1	115.4
1934	71.6	59.0	53.9	67.6	78.9	94.2	93.6	88.5	114.2
1934									
Jan.	70.6	55.3	47.9	67.8	78.2	86.8	84.5	48.2	108.1
Feb.	72.1	58.0	49.3	72.5	78.7	86.4	84.0	67.1	98.6
Mar.	72.0	56.5	49.5	68.3	79.9	93.1	92.0	63.8	97.0
Apr.	71.1	55.4	48.7	66.6	79.4	92.6	91.4	56.9	94.5
May	71.1	56.9	51.1	66.5	78.5	99.6	99.4	130.6	102.6
June	72.1	59.3	55.5	65.6	78.2	95.8	95.2	97.2	126.1
July	72.0	60.0	57.8	63.7	78.4	95.7	95.6	148.8	116.3
Aug.	72.3	61.6	60.7	63.1	78.7	99.0	99.8	172.8	114.7
Sept.	72.0	61.3	58.9	65.3	79.0	97.1	97.5	127.7	117.7
Oct.	71.4	60.9	55.3	70.4	79.3	95.8	95.3	61.2	128.8
Nov.	71.2	61.2	55.7	70.4	79.4	96.5	97.0	51.2	130.4
Dec.	71.2	61.6	56.0	70.9	79.0	92.4	91.0	36.0	135.7
1935									
Jan.	71.5	61.4	55.7	71.0	78.9	97.5	97.8	30.6	143.7
Feb.	71.9	62.0	55.7	72.6	79.1	100.6	101.1	62.2	141.2
Mar.	72.0	62.7	56.4	73.3	79.0	94.2	93.3	65.4	143.2
Apr.	72.5	64.7	59.8	72.9	78.8	98.3	97.7	91.8	135.8
May	72.3	64.1	58.0	74.4	78.8	103.2	104.4	86.3	123.2

1. See Prices and Price Indexes 1913-1928, pp. 19-21, 270-289 and 1913-1933, p. 15.

2. Wholesale prices of Canadian products of farm origin only See Prices and Price Indexes 1913-1933, p. 33, and Monthly Mimeographs 1934 and 1935.

3. Wholesale prices of grains, fruits and vegetables.

4. Wholesale prices of Animals and Animal Products.

5. Including foods, rents, fuel, clothing and sundries, See Prices and price Indexes 1913-1928, pp. 181-185, 290-293. 1926 = 100.

Prices and Price Indexes 1913-1931, p. 108, and Monthly Mimeographs 1934-1935.

6. Monthly Review of Business Statistics, p. 8, and Monthly Indexes of the Physical volume of business in Canada, supplement to the Monthly Review of Business Statistics, November, 1932.

General Situation.—The economic situation in general during the first five months of 1935 has been characterized by comparative stability of wholesale prices as a whole. Prices of Canadian farm products have tended toward higher levels except during the month of May. Prices of field products have shown the most marked decline. Although the index of prices of animal products has varied more

from month to month, the trend has been definitely upward. Retail prices have largely reflected the condition of the wholesale market, such variations as have taken place have been relatively small. The physical volume of business has varied as much as nine points, the peaks being reached in February and May. Probably the weakest points in the whole economic position of the country at the present time are the continued slackness in construction industries and the comparatively light movement of wheat into export markets. In regard to the latter it is now evident that the carryover at the end of July will be much larger than was anticipated earlier in the crop year. Exports in general have been much higher than in the corresponding months of 1934.

Compared with a year ago the index of wholesale prices is about one point higher. There has, however, been more substantial improvement in prices of Canadian farm products. The total index in May 1935, was about five points above that for May, 1934. In spite of the decline in May of this year the index of prices of field products was still nearly 7 points higher than a year ago and in the case of animal products the gain has been about 9 points. The physical volume of production and industrial production are both well above the position reached in May, 1934 Collections appear to be better and optimism more general. Progress toward recovery has been substantial even though not as rapid as could be wished for or as general as desired.

LA SITUATION ÉCONOMIQUE

PRÉPARÉ PAR LA DIVISION DE L'ÉCONOMIE AGRICOLE, MINISTÈRE DE L'AGRICULTURE, OTTAWA, PRINCIPALEMENT AVEC LES DONNÉES COMPILÉES AU BUREAU FÉDÉRAL DE LA STATISTIQUE

Le chiffre indice des prix du gros au Canada a subi un léger recul en mai, principalement à cause de la baisse de prix enregistrée sur le blé et sur l'argent. L'indice des produits végétaux, qui était à 69·4 en avril est tombé à 68·0 en mai. Il y a eu régression également chez les minéraux non métalliques et les produits chimiques. C'est le groupe de métaux non ferreux qui a enregistré la plus forte hausse, de 67·9 à 70·7. Dans l'ensemble cependant, les prix étaient plus bas et l'indice des prix du gros est tombé à 72·3.

Prix du détail.—L'indice des prix du détail et des frais de services est resté stationnaire en mai. Les prix des aliments ont un peu remonté, de même que l'indice des loyers, qui n'est computé qu'en octobre et mai. Les prix du combustible, qui sont allés en diminuant toute la saison, étaient inférieurs à ceux de l'année dernière. En général, les prix du détail en mai 1935 n'étaient qu'un peu plus élevés qu'en mai 1934.

Volume physique des affaires.—Le volume physique des affaires a atteint un nouveau point élevé dans la période de redressement; il était à 103·2 contre 100 en 1926 et 98·3 en avril de cette année. L'indice de la production industrielle est monté de 97·7 à 104·4. La production minérale était inférieure à celle d'avril, malgré l'augmentation sensible enregistrée dans les exportations de nickel et d'argent, les importations de bauxite et la production du charbon. L'indice des produits manufacturés s'est élevé de 94·0 à 105·1. Presque toutes les industries comprises dans ce groupe ont fait preuve d'une activité croissante, mais la production du fer et de l'acier était un peu moins forte que dans le mois précédent et la production des automobiles est tombée de 102·9 à 87. L'indice du bâtiment était de 38·1 contre 37·1. Le nombre de contrats passés était plus élevé mais les permis de construction avaient une moindre valeur et le coût de la construction est resté stationnaire. Les chargements de wagons étaient un peu moins nombreux, mais les exportations et les importations ont remonté. Les facteurs agricoles sont discutés en détail dans un autre paragraphe. Notons cependant que les ventes agricoles et les stocks entreposés au froid étaient moins élevés en mai qu'en avril.

Produits agricoles.—Le chiffre indice des prix du gros des produits de ferme canadiens est tombé à 64·1 en mai. Il y a eu une hausse très prononcée dans les prix des produits animaux, l'indice s'est élevé de 72·9 en avril à 74·4 en mai. Les prix des porcs et des agneaux ont beaucoup remonté à cause des expéditions relativement faibles. Les prix des bœufs, bons à de choix, ont remonté pendant la première moitié du mois à Toronto et à Winnipeg, mais ils sont retombés plus tard à cause de la faiblesse des prix aux Etats-Unis. Les prix des veaux ont diminué à Toronto et à Winnipeg. L'indice des prix des produits des champs, qui était à 59·8 en avril, est tombé à 58·0 en mai. Le prix moyen du blé du Nord Manitoba N° 1 est à peu près de deux cents inférieur à celui d'avril, et le prix de l'avoine a baissé également, mais un peu moins. Le prix moyen de l'orge C.O. N° 2 est tombé de 45·9 à 42·3 cents. Le lin C.O. N° 1 est tombé de 140·9 à 134·0. La récolte de blé de l'Ouest du Canada est plus abondante que celle de l'année dernière. On dit que les prévisions sont très bonnes dans le Sud de la Saskatchewan et que l'on prévoit une augmentation dans la récolte de fourrages en dépit de la sécheresse excessive qui a sévi dans certains secteurs de l'Alberta et de la Saskatchewan. Il y a cependant des districts où la récolte est en retard et où la température jouera un grand rôle dans le succès de la moisson.

L'indice des ventes de grain, qui était à 91·8 en avril est tombé à 86·3 en mai. Les expéditions de blé, d'avoine, d'orge, de seigle et de lin ont diminué. L'indice des expéditions de blé est tombé de plus de sept points, celui de l'avoine de près de neuf points, et celui des ventes d'orge d'environ trois points par rapport à avril.

Les ventes totales de bestiaux n'ont été que légèrement inférieures à celles d'avril et la baisse de l'indice n'a pas atteint deux points. La diminution la plus forte a été dans l'expédition de moutons et d'agneaux. Cet indice est basé sur les livraisons aux marchés publics.

Situation générale.—En général la situation économique pendant les cinq premiers mois de 1935 s'est caractérisée par une stabilité relative des prix du gros. Les prix des produits de ferme canadiens avaient aussi une tendance à la hausse, sauf pendant le mois de mai. Ce sont les prix des produits des champs qui ont le plus baissé. L'indice des prix des produits animaux a varié d'un mois à l'autre, mais la tendance était nettement à la hausse. Les prix du détail réfléchissaient principalement l'état du marché de gros, et les variations qui se sont produites ont été relativement faibles. Le volume physique des affaires a varié d'au moins neuf points, le maximum étant atteint en février et mai. Les points les plus faibles peut-être de la situation économique du pays à l'heure actuelle sont l'inertie continuelle de l'industrie du bâtiment et les expéditions relativement légères de blé sur les marchés d'exportation. En ce qui concerne ce produit, il est maintenant évident que le reliquat à la fin de juillet sera beaucoup plus fort que l'on ne prévoyait au commencement de l'année de récolte. Les exportations en général ont été plus fortes que dans les mois correspondants de 1934.

L'indice des prix du gros est d'environ un point plus élevé que l'année dernière, mais l'amélioration constatée dans les prix des produits de ferme canadiens a été considérable. L'indice total en mai 1935 était d'environ cinq points au-dessus de celui de mai 1934. Malgré la baisse notée en mai de cette année, l'indice des prix des produits des champs était encore à peu près de 7 points plus élevé que l'année dernière, et l'augmentation était de près de 9 points pour les produits animaux. Le volume physique de la production et la production industrielle sont tous deux bien au-dessus du point qu'ils occupaient en mai 1934. Il semble que les recouvrements se font mieux et l'optimisme est plus général. Il s'est fait évidemment de grands progrès dans la voie du redressement quoique ces progrès n'aient pas été aussi rapides ni aussi étendus qu'on aurait pu le désirer.